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(54) Title: <i>CHLAMYDIA</i> ANTIGENS AND CORRESPONDING DNA FRAGMENTS AND USES THEREOF		
(57) Abstract <p>The present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of <i>Chlamydia</i>, specifically <i>C. pneumoniae</i>, employing a vector containing a nucleotide sequence encoding full-length, 5'-truncated or 3'-truncated 76kDa protein of a strain of <i>Chlamydia pneumoniae</i> and a promoter to effect expression of the 76kDa protein gene in the host. Modifications are possible within the scope of this invention.</p>		

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TITLE OF INVENTION

CHLAMYDIA ANTIGENS AND CORRESPONDING DNA FRAGMENTS
AND USES THEREOF

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REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S.
Provisional Application No. 60/132,270, filed May 3, 1999, and
10 U.S. Provisional Application No. 60/141,276 filed June 30,
1999.

FIELD OF INVENTION

15 The present invention relates to the *Chlamydia* 76kDa
protein and corresponding DNA molecules, which can be used to
prevent and treat *Chlamydia* infection in mammals, such as
humans.

20

BACKGROUND OF THE INVENTION

Chlamydiae are prokaryotes. They exhibit morphologic
and structural similarities to gram-negative bacteria including
a trilaminar outer membrane, which contains lipopolysaccharide
25 and several membrane proteins that are structurally and
functionally analogous to proteins found in *E coli*. They are
obligate intra-cellular parasites with a unique biphasic life
cycle consisting of a metabolically inactive but infectious
extracellular stage and a replicating but non-infectious
30 intracellular stage. The replicative stage of the life-cycle
takes place within a membrane-bound inclusion which sequesters
the bacteria away from the cytoplasm of the infected host cell.

C. pneumoniae is a common human pathogen, originally
described as the TWAR strain of *Chlamydia psittaci* but
35 subsequently recognised to be a new species. *C. pneumoniae* is

antigenically, genetically and morphologically distinct from other Chlamydia species (*C. trachomatis*, *C. pecorum* and *C. psittaci*). It shows 10% or less DNA sequence homology with either of *C. trachomatis* or *C. psittaci*.

5 *C. pneumoniae* is a common cause of community acquired pneumonia, only less frequent than *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Campos et al. (1995) Investigation of Ophthalmology and Visual Science 36:1477). It
10 can also cause upper respiratory tract symptoms and disease, including bronchitis and sinusitis (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Grayston et al (1990) Journal of Infectious Diseases 161:618; Marrie (1993) Clinical Infectious Diseases. 18:501; Wang et al (1986) Chlamydial
15 infections Cambridge University Press, Cambridge. p. 329. The great majority of the adult population (over 60%) has antibodies to *C. pneumoniae* (Wang et al (1986) Chlamydial infections. Cambridge University Press, Cambridge. p. 329), indicating past infection which was unrecognized or
20 asymptomatic.

C. pneumoniae infection usually presents as an acute respiratory disease (i.e., cough, sore throat, hoarseness, and fever; abnormal chest sounds on auscultation). For most
25 patients, the cough persists for 2 to 6 weeks, and recovery is slow. In approximately 10% of these cases, upper respiratory tract infection is followed by bronchitis or pneumonia. Furthermore, during a *C. pneumoniae* epidemic, subsequent co-infection with pneumococcus has been noted in about half of these pneumonia patients, particularly in the infirm and the
30 elderly. As noted above, there is more and more evidence that *C. pneumoniae* infection is also linked to diseases other than respiratory infections.

The reservoir for the organism is presumably people. In contrast to *C. psittaci* infections, there is no known bird

or animal reservoir. Transmission has not been clearly defined. It may result from direct contact with secretions, from fomites, or from airborne spread. There is a long incubation period, which may last for many months. Based on
5 analysis of epidemics, *C. pneumoniae* appears to spread slowly through a population (case-to-case interval averaging 30 days) because infected persons are inefficient transmitters of the organism. Susceptibility to *C. pneumoniae* is universal. Reinfections occur during adulthood, following the primary
10 infection as a child. *C. pneumoniae* appears to be an endemic disease throughout the world, noteworthy for superimposed intervals of increased incidence (epidemics) that persist for 2 to 3 years. *C. trachomatis* infection does not confer cross-immunity to *C. pneumoniae*. Infections are easily treated with
15 oral antibiotics, tetracycline or erythromycin (2 g/d, for at least 10 to 14 d). A recently developed drug, azithromycin, is highly effective as a single-dose therapy against Chlamydial infections.

In most instances, *C. pneumoniae* infection is often
20 mild and without complications, and up to 90% of infections are subacute or unrecognized. Among children in industrialized countries, infections have been thought to be rare up to the age of 5 y, although a recent study (E Normann et al, Chlamydia pneumoniae in children with acute respiratory tract infections,
25 Acta Paediatrica, 1998, Vol 87, Iss 1, pp 23-27) has reported that many children in this age group show PCR evidence of infection despite being seronegative, and estimates a prevalence of 17-19% in 2-4 y olds. In developing countries, the seroprevalence of *C. pneumoniae* antibodies among young
30 children is elevated, and there are suspicions that *C. pneumoniae* may be an important cause of acute lower respiratory tract disease and mortality for infants and children in tropical regions of the world.

From seroprevalence studies and studies of local epidemics, the initial *C. pneumoniae* infection usually happens between the ages of 5 and 20 y. In the USA, for example, there are estimated to be 30,000 cases of childhood pneumonia each year caused by *C. pneumoniae*. Infections may cluster among groups of children or young adults (e.g., school pupils or military conscripts).

C. pneumoniae causes 10 to 25% of community-acquired lower respiratory tract infections (as reported from Sweden, Italy, Finland, and the USA). During an epidemic, *C. pneumoniae* infection may account for 50 to 60% of the cases of pneumonia. During these periods, also, more episodes of mixed infections with *S. pneumoniae* have been reported.

Reinfection during adulthood is common; the clinical presentation tends to be milder. Based on population seroprevalence studies, there tends to be increased exposure with age, which is particularly evident among men. Some investigators have speculated that a persistent, asymptomatic *C. pneumoniae* infection state is common.

In adults of middle age or older, *C. pneumoniae* infection may progress to chronic bronchitis and sinusitis. A study in the USA revealed that the incidence of pneumonia caused by *C. pneumoniae* in persons younger than 60 years is 1 case per 1,000 persons per year; but in the elderly, the disease incidence rose three-fold. *C. pneumoniae* infection rarely leads to hospitalization, except in patients with an underlying illness.

Of considerable importance is the association of atherosclerosis and *C. pneumoniae* infection. There are several epidemiological studies showing a correlation of previous infections with *C. pneumoniae* and heart attacks, coronary artery and carotid artery disease (Saikku et al. (1988) Lancet;ii:983; Thom et al. (1992) JAMA 268:68; Linnanmaki et al. (1993), Circulation 87:1030; Saikku et al. (1992) Annals

Internal Medicine 116:273; Melnick et al (1993) American Journal of Medicine 95:499). Moreover, the organisms has been detected in atheromas and fatty streaks of the coronary, carotid, peripheral arteries and aorta (Shor et al. (1992) South African. Medical Journal 82:158; Kuo et al. (1993) Journal of Infectious Diseases 167:841; Kuo et al. (1993) Arteriosclerosis and Thrombosis 13:1500; Campbell et al (1995) Journal of Infectious Diseases 172:585; Chiu et al. Circulation, 1997 (In Press)). Viable *C. pneumoniae* has been recovered from the coronary and carotid artery (Ramirez et al (1996) Annals of Internal Medicine 125:979; Jackson et al. Abst. K121, p272, 36th ICAAC, 15-18 Sept. 1996, New Orleans). Furthermore, it has been shown that *C. pneumoniae* can induce changes of atherosclerosis in a rabbit model (Fong et al (1997) Journal of Clinical Microbiology 35:48). Taken together, these results indicate that it is highly probable that *C. pneumoniae* can cause atherosclerosis in humans, though the epidemiological importance of Chlamydial atherosclerosis remains to be demonstrated.

20 A number of recent studies have also indicated an association between *C. pneumoniae* infection and asthma. Infection has been linked to wheezing, asthmatic bronchitis, adult-onset asthma and acute exacerbations of asthma in adults, and small-scale studies have shown that prolonged antibiotic treatment was effective at greatly reducing the severity of the disease in some individuals (Hahn DL, et al. Evidence for Chlamydia pneumoniae infection in steroid-dependent asthma. Ann Allergy Asthma Immunol. 1998 Jan; 80(1): 45-49.; Hahn DL, et al. Association of Chlamydia pneumoniae IgA antibodies with recently symptomatic asthma. Epidemiol Infect. 1996 Dec; 117(3): 513-517; Bjornsson E, et al. Serology of Chlamydia in relation to asthma and bronchial hyperresponsiveness. Scand J Infect Dis. 1996; 28(1): 63-69.; Hahn DL. Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before-after

trial. J Fam Pract. 1995 Oct; 41(4): 345-351.; Allegra L, et al. Acute exacerbations of asthma in adults: role of Chlamydia pneumoniae infection. Eur Respir J. 1994 Dec; 7(12): 2165-2168.; Hahn DL, et al. Association of Chlamydia pneumoniae
5 (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA. 1991 Jul 10; 266(2): 225-230).

In light of these results a protective vaccine against *C. pneumoniae* infection would be of considerable importance. There is not yet an effective vaccine for any
10 human Chlamydial infection. It is conceivable that an effective vaccine can be developed using physically or chemically inactivated Chlamydiae. However, such a vaccine does not have a high margin of safety. In general, safer vaccines are made by genetically manipulating the organism by
15 attenuation or by recombinant means. Accordingly, a major obstacle in creating an effective and safe vaccine against human Chlamydial infection has been the paucity of genetic information regarding Chlamydia, specifically *C. pneumoniae*.

Studies with *C. trachomatis* and *C. psittaci* indicate
20 that safe and effective vaccine against Chlamydia is an attainable goal. For example, mice which have recovered from a lung infection with *C. trachomatis* are protected from infertility induced by a subsequent vaginal challenge (Pal et al.(1996) Infection and Immunity.64:5341). Similarly, sheep
25 immunized with inactivated *C. psittaci* were protected from subsequent Chlamydial-induced abortions and stillbirths (Jones et al. (1995) Vaccine 13:715). Protection from Chlamydial infections has been associated with Th1 immune responses, particularly the induction of INF γ - producing CD4+T-cells
30 (Igietsemes et al. (1993) Immunology 5:317). The adoptive transfer of CD4+ cell lines or clones to nude or SCID mice conferred protection from challenge or cleared chronic disease (Igietseme et al (1993) Regional Immunology 5:317; Magee et al (1993) Regional Immunology 5: 305), and *in vivo* depletion of

CD4+ T cells exacerbated disease post-challenge (Landers et al (1991) Infection & Immunity 59:3774; Magee et al (1995) Infection & Immunity 63:516). However, the presence of sufficiently high titres of neutralising antibody at mucosal
5 surfaces can also exert a protective effect (Cotter et al. (1995) Infection and Immunity 63:4704).

Antigenic variation within the species *C. pneumoniae* is not well documented due to insufficient genetic information, though variation is expected to exist based on *C. trachomatis*.
10 Serovars of *C. trachomatis* are defined on the basis of antigenic variation in the major outer membrane protein (MOMP), but published *C. pneumoniae* MOMP gene sequences show no variation between several diverse isolates of the organism (Campbell et al (1990) Infection and Immunity 58:93; McCafferty
15 et al (1995) Infection and Immunity 63:2387-9; Knudsen et al (1996) Third Meeting of the European Society for Chlamydia Research, Vienna). Melgosa et al. (Infect. Immun. 1994. 62:880) claimed to have cloned the gene encoding a 76 kDa antigen from a single strain of *C. pneumoniae*. An operon
20 encoding the 9 kDa and 9kDa cyteine-rich outer membrane protein genes has been described (Watson et al., Nucleic Acids Res (1990) 18:5299; Watson et al., Microbiology (1995) 141:2489). Many antigens recognized by immune sera to *C. pneumoniae* are conserved across all Chlamydiae, but 98 kDa, 76 kDa and several
25 other proteins may be *C. pneumoniae*-specific (Perez Melgosa et al., Infect. Immun. 1994. 62:880; Melgosa et al., FEMS Microbiol Lett (1993) 112 :199;; Campbell et al., J Clin Microbiol (1990) 28 :1261; Iijima et al., J Clin Microbiol (1994) 32:583). An assessment of the number and relative
30 frequency of any *C. pneumoniae* serotypes, and the defining antigens, is not yet possible. The entire genome sequence of *C. pneumoniae* strain CWL-029 is now known ([http://chlamydia-
www.berkeley.edu:4231/](http://chlamydia-
www.berkeley.edu:4231/)) and as further sequences become

available a better understanding of antigenic variation may be gained.

Many antigens recognised by immune sera to *C. pneumoniae* are conserved across all Chlamydiae, but 98kDa, 76 kDa and 54 kDa proteins appear to be *C. pneumoniae*-specific (Campos et al. (1995) Investigation of Ophthalmology and Visual Science 36:1477; Marrie (1993) Clinical Infectious Diseases. 18:501; Wiedmann-Al-Ahmad M, et al. Reactions of polyclonal and neutralizing anti-p54 monoclonal antibodies with an isolated, species-specific 54-kilodalton protein of Chlamydia pneumoniae. Clin Diagn Lab Immunol. 1997 Nov; 4(6): 700-704).

Immunoblotting of isolates with sera from patients does show variation of blotting patterns between isolates, indicating that serotypes *C. pneumoniae* may exist (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Ramirez et al (1996) Annals of Internal Medicine 125:979). However, the results are potentially confounded by the infection status of the patients, since immunoblot profiles of a patient's sera change with time post-infection. An assessment of the number and relative frequency of any serotypes, and the defining antigens, is not yet possible.

Accordingly, a need exists for identifying and isolating polynucleotide sequences of *C. pneumoniae* for use in preventing and treating Chlamydia infection.

25

SUMMARY OF THE INVENTION

The present invention provides purified and isolated polynucleotide molecules that encode the *Chlamydia* polypeptide designated 76kDa protein (SEQ ID No: 1) which can be used in methods to prevent, treat, and diagnose *Chlamydia* infection. In one form of the invention, the polynucleotide molecules are DNA that encode the polypeptide of SEQ ID No: 2.

Another form of the invention provides polypeptides corresponding to the isolated DNA molecules. The amino acid sequence of the corresponding encoded polypeptide is shown as SEQ ID No: 2.

5 Another form of the invention provides truncated polypeptides corresponding to truncated DNA molecules. In one embodiment, the truncated nucleotide and amino acid sequences are shown as SEQ ID Nos: 3 and 4 respectively. In another embodiment, the truncated nucleotide and amino acid sequences
10 are shown as SEQ ID Nos: 5 and 6 respectively.

Although Melgosa et al. has reported cloning a 76kDa protein from *C. pneumoniae*, comparison of the gene sequence as reported by Melgosa et al. to the published genome sequence of *C. pneumoniae* (<http://chlamydia-www.berkeley.edu:4231/>) reveals
15 that, in fact, the genomic sequence in this region contains at least two open reading frames (ORFs), one in the 5' portion and one in the 3' portion. The sequence reported in Melgosa et al. is an in-frame fusion of the 5' end of the 5' ORF. Thus, Melgosa's deduced protein is merely a 76kDa fusion protein and
20 not the 76kDa protein observed by immunoblotting from various *C. pneumoniae* isolates. By contrast, the 76kDa protein of the present invention is the full-length protein encoded by the 3'ORF in this region of the genome. Notably, further analysis of the genome sequence ([http://chlamydia-](http://chlamydia-www.berkeley.edu:4231/)
25 [www.berkeley.edu:4231/](http://chlamydia-www.berkeley.edu:4231/)) reveals at least one in-frame ATG upstream of the start codon of the 5' ORF, suggesting that the 5' ORF may form part of one or more larger ORFs.

Those skilled in the art will readily understand that the invention, having provided the polynucleotide sequences
30 encoding the *Chlamydia* 76kDa protein, also provides polynucleotides encoding fragments derived from such a polypeptide. Moreover, the invention is understood to provide mutants and derivatives of such polypeptides and fragments derived therefrom, which result from the addition, deletion, or

substitution of non-essential amino acids as described herein. Those skilled in the art would also readily understand that the invention, having provided the polynucleotide sequences encoding *Chlamydia* polypeptides, further provides monospecific
5 antibodies that specifically bind to such polypeptides.

The present invention has wide application and includes expression cassettes, vectors, and cells transformed or transfected with the polynucleotides of the invention. Accordingly, the present invention further provides (i) a
10 method for producing a polypeptide of the invention in a recombinant host system and related expression cassettes, vectors, and transformed or transfected cells; (ii) a vaccine, or a live vaccine vector such as a pox virus, *Salmonella typhimurium*, or *Vibrio cholerae* vector, containing a
15 polynucleotide of the invention, such vaccines and vaccine vectors being useful for, e.g., preventing and treating *Chlamydia* infection, in combination with a diluent or carrier, and related pharmaceutical compositions and associated therapeutic and/or prophylactic methods; (iii) a therapeutic
20 and/or prophylactic use of an RNA or DNA molecule of the invention, either in a naked form or formulated with a delivery vehicle, a polypeptide or combination of polypeptides, or a monospecific antibody of the invention, and related pharmaceutical compositions; (iv) a method for diagnosing the
25 presence of *Chlamydia* in a biological sample, which can involve the use of a DNA or RNA molecule, a monospecific antibody, or a polypeptide of the invention; and (v) a method for purifying a polypeptide of the invention by antibody-based affinity chromatography.

30

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be further understood from the following description with reference to embodiments shown in the drawings, in which:

Figure 1 shows the full-length nucleotide sequence of the 76kDa protein gene (SEQ ID No: 1) and the deduced amino acid sequence of the 76kDa protein from *Chlamydia pneumoniae* (SEQ ID No: 2).

Figure 2 shows the restriction enzyme analysis of the *C. pneumoniae* 76kDa protein gene.

Figure 3 shows the nucleotide sequence containing a 3'-truncated 76kDa protein gene and its corresponding deduced amino acid sequence from *Chlamydia pneumoniae*; (note that nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76kDa protein gene).

Figure 4 shows the construction and elements of plasmid pCACPNM555a, containing the full-length 76kDa gene.

Figure 5 shows the construction and elements of plasmid pCAI555, containing a 5'-truncated version of the 76kDa gene.

Figure 6 shows the construction and elements of plasmid pCAD76kDa, containing a 3'-truncated version of the 76kDa gene from Figure 3.

Figure 7 illustrates protection against *C. pneumoniae* infection by pCACPNM555a following DNA immunization.

Figure 8 illustrates protection against *C. pneumoniae* infection by pCAI555 following DNA immunization.

Figure 9 illustrates protection against *C. pneumoniae* infection by pCAD76kDa following DNA immunization. For Figures 7 to 9, individual data points are shown for each animal (hollow diamonds) as well as mean and standard deviations for each group (solid squares).

DETAILED DESCRIPTION OF INVENTION

The invention is described with reference to the
5 following sequences which are embodiments of the invention:
SEQ ID NO: 1 is the full-length sequence of the 76kDa protein
gene.

SEQ ID NO: 2 is the deduced full-length amino acid
sequence of the 76kDa protein.

10 SEQ ID NO: 3 is the 5'-truncated nucleotide sequence
of the 76kDa protein gene.

SEQ ID NO: 4 is the 5'-truncated amino acid sequence
of the 76kDa protein.

15 SEQ ID NO: 5 is the 3'-truncated nucleotide sequence
of the 76kDa protein gene.

SEQ ID NO: 6 is the 3'-truncated amino acid sequence
of the 76kDa protein, which forms the basis for
immunoprotection by pCAD76kDa in Figure 9.

20 SEQ ID NO: 7 is the sequence encoding a polypeptide
containing a truncated 76kDa protein. Using this sequence as a
template, a fragment was amplified by PCR to form part of
construct pCAD76kDa.

SEQ ID NO: 8 is the deduced amino acid sequence of a
truncated 76kDa protein, as expressed from pCAD76kDa.

25 SEQ ID NO: 9 is the 5' primer used to clone the full-
length 76kDa protein gene and to amplify the full-length 76kDa
protein gene for pCACPNM555a.

30 SEQ ID NO: 10 is the 3' primer used to clone the
full-length 76kDa protein gene and to amplify the full-length
76kDa protein gene for pCACPNM555a.

SEQ ID NO: 11 is the 5' primer used to amplify the
5'-truncated 76kDa protein gene fragment for pCAI555.

SEQ ID NO: 12 is the 3' primer used to amplify the
5'-truncated 76kDa protein gene fragment for pCAI555.

SEQ ID NO: 13 is the 5' primer used to amplify the 3'-truncated 76kDa protein gene fragment for pCAD76kDa.

SEQ ID NO: 14 is the 3' primer used to amplify the truncated 76kDa protein gene fragment for pCAD76kDa.

5 An open reading frame (ORF) encoding the Chlamydial 76kDa protein has been identified from the *C. pneumoniae* genome. The gene encoding this protein and its fragments have been inserted into expression plasmids and shown to confer immune protection against Chlamydial infection. Accordingly,
10 this 76kDa protein and related polypeptides can be used to prevent and treat *Chlamydia* infection.

 According to a first aspect of the invention, isolated polynucleotides are provided which encode *Chlamydia* polypeptides, whose amino acid sequences are shown in SEQ ID
15 Nos: 2, 4 and 6.

 The term "isolated polynucleotide" is defined as a polynucleotide removed from the environment in which it naturally occurs. For example, a naturally-occurring DNA molecule present in the genome of a living bacteria or as part
20 of a gene bank is not isolated, but the same molecule separated from the remaining part of the bacterial genome, as a result of, e.g., a cloning event (amplification), is isolated. Typically, an isolated DNA molecule is free from DNA regions (e.g., coding regions) with which it is immediately contiguous
25 at the 5' or 3' end, in the naturally occurring genome. Such isolated polynucleotides may be part of a vector or a composition and still be defined as isolated in that such a vector or composition is not part of the natural environment of such polynucleotide.

30 The polynucleotide of the invention is either RNA or DNA (cDNA, genomic DNA, or synthetic DNA), or modifications, variants, homologs or fragments thereof. The DNA is either double-stranded or single-stranded, and, if single-stranded, is either the coding strand or the non-coding (anti-sense) strand.

Any one of the sequences that encode the polypeptides of the invention as shown in SEQ ID No: 1, 3 or 5 is (a) a coding sequence, (b) a ribonucleotide sequence derived from transcription of (a), or (c) a coding sequence which uses the
5 redundancy or degeneracy of the genetic code to encode the same polypeptides. By "polypeptide" or "protein" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Both terms are used interchangeably in the present application.

10 Consistent with the first aspect of the invention, amino acid sequences are provided which are homologous to SEQ ID No: 2, 4 or 6. As used herein, "homologous amino acid sequence" is any polypeptide which is encoded, in whole or in part, by a nucleic acid sequence which hybridizes at 25-35°C
15 below critical melting temperature (T_m), to any portion of the nucleic acid sequence of SEQ ID No: 1, 3 or 5. A homologous amino acid sequence is one that differs from an amino acid sequence shown in SEQ ID No: 2, 4 or 6 by one or more conservative amino acid substitutions. Such a sequence also
20 encompass serotypic variants (defined below) as well as sequences containing deletions or insertions which retain inherent characteristics of the polypeptide such as immunogenicity. Preferably, such a sequence is at least 75%, more preferably 80%, and most preferably 90% identical to SEQ
25 ID No: 2, 4 or 6.

Homologous amino acid sequences include sequences that are identical or substantially identical to SEQ ID No: 2, 4 or 6. By "amino acid sequence substantially identical" is meant a sequence that is at least 90%, preferably 95%, more
30 preferably 97%, and most preferably 99% identical to an amino acid sequence of reference and that preferably differs from the sequence of reference by a majority of conservative amino acid substitutions.

Conservative amino acid substitutions are substitutions among amino acids of the same class. These classes include, for example, amino acids having uncharged polar side chains, such as asparagine, glutamine, serine, threonine, and tyrosine; amino acids having basic side chains, such as lysine, arginine, and histidine; amino acids having acidic side chains, such as aspartic acid and glutamic acid; and amino acids having nonpolar side chains, such as glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and cysteine.

Homology is measured using sequence analysis software such as Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705. Amino acid sequences are aligned to maximize identity. Gaps may be artificially introduced into the sequence to attain proper alignment. Once the optimal alignment has been set up, the degree of homology is established by recording all of the positions in which the amino acids of both sequences are identical, relative to the total number of positions.

Homologous polynucleotide sequences are defined in a similar way. Preferably, a homologous sequence is one that is at least 45%, more preferably 60%, and most preferably 85% identical to the coding sequence of SEQ ID No: 1, 3 or 5.

Consistent with the first aspect of the invention, polypeptides having a sequence homologous to SEQ ID No: 2, 4 or 6 include naturally-occurring allelic variants, as well as mutants or any other non-naturally occurring variants that retain the inherent characteristics of the polypeptide of SEQ ID No: 2, 4 or 6.

As is known in the art, an allelic variant is an alternate form of a polypeptide that is characterized as having a substitution, deletion, or addition of one or more amino acids that does not alter the biological function of the

polypeptide. By "biological function" is meant the function of the polypeptide in the cells in which it naturally occurs, even if the function is not necessary for the growth or survival of the cells. For example, the biological function of a porin is to allow the entry into cells of compounds present in the extracellular medium. Biological function is distinct from antigenic property. A polypeptide can have more than one biological function.

Allelic variants are very common in nature. For example, a bacterial species such as *C. pneumoniae*, is usually represented by a variety of strains that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfills the same biological function in different strains can have an amino acid sequence (and polynucleotide sequence) that is not identical in each of the strains. Despite this variation, an immune response directed generally against many allelic variants has been demonstrated. In studies of the *Chlamydial* MOMP antigen, cross-strain antibody binding plus neutralization of infectivity occurs despite amino acid sequence variation of MOMP from strain to strain, indicating that the MOMP, when used as an immunogen, is tolerant of amino acid variations.

Polynucleotides encoding homologous polypeptides or allelic variants are retrieved by polymerase chain reaction (PCR) amplification of genomic bacterial DNA extracted by conventional methods. This involves the use of synthetic oligonucleotide primers matching upstream and downstream of the 5' and 3' ends of the encoding domain. Suitable primers are designed according to the nucleotide sequence information provided in SEQ ID No:1, 3 or 5. The procedure is as follows: a primer is selected which consists of 10 to 40, preferably 15 to 25 nucleotides. It is advantageous to select primers containing C and G nucleotides in a proportion sufficient to ensure efficient hybridization; i.e., an amount of C and G

nucleotides of at least 40%, preferably 50% of the total nucleotide content. A standard PCR reaction contains typically 0.5 to 5 Units of Taq DNA polymerase per 100 μ L, 20 to 200 μ M deoxynucleotide each, preferably at equivalent concentrations, 5 0.5 to 2.5 mM magnesium over the total deoxynucleotide concentration, 10^5 to 10^6 target molecules, and about 20 pmol of each primer. About 25 to 50 PCR cycles are performed, with an annealing temperature 15°C to 5°C below the true T_m of the primers. A more stringent annealing temperature improves 10 discrimination against incorrectly annealed primers and reduces incorporation of incorrect nucleotides at the 3' end of primers. A denaturation temperature of 95°C to 97°C is typical, although higher temperatures may be appropriate for dematuration of G+C-rich targets. The number of cycles 15 performed depends on the starting concentration of target molecules, though typically more than 40 cycles is not recommended as non-specific background products tend to accumulate.

An alternative method for retrieving polynucleotides 20 encoding homologous polypeptides or allelic variants is by hybridization screening of a DNA or RNA library. Hybridization procedures are well-known in the art and are described in Ausubel et al., (Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994), Silhavy et al. (Silhavy 25 et al. Experiments with Gene Fusions, Cold Spring Harbor Laboratory Press, 1984), and Davis et al. (Davis et al. A Manual for Genetic Engineering: Advanced Bacterial Genetics, Cold Spring Harbor Laboratory Press, 1980)). Important parameters for optimizing hybridization conditions are 30 reflected in a formula used to obtain the critical melting temperature above which two complementary DNA strands separate from each other (Casey & Davidson, Nucl. Acid Res. (1977) 4:1539). For polynucleotides of about 600 nucleotides or

larger, this formula is as follows: $T_m = 81.5 + 0.41 \times (\% \text{ G+C}) + 16.6 \log (\text{cation ion concentration}) - 0.63 \times (\% \text{ formamide}) - 600/\text{base number}$. Under appropriate stringency conditions, hybridization temperature (T_h) is approximately 20 to 40°C, 20
5 to 25°C, or, preferably 30 to 40°C below the calculated T_m . Those skilled in the art will understand that optimal temperature and salt conditions can be readily determined.

For the polynucleotides of the invention, stringent conditions are achieved for both pre-hybridizing and
10 hybridizing incubations (i) within 4-16 hours at 42°C, in 6 x SSC containing 50% formamide, or (ii) within 4-16 hours at 65°C in an aqueous 6 x SSC solution (1 M NaCl, 0.1 M sodium citrate (pH 7.0)). Typically, hybridization experiments are performed at a temperature from 60 to 68°C, e.g. 65°C. At such a
15 temperature, stringent hybridization conditions can be achieved in 6xSSC, preferably in 2xSSC or 1xSSC, more preferably in 0.5xSSC, 0.3xSSC or 0.1xSSC (in the absence of formamide). 1xSSC contains 0.15 M NaCl and 0.015 M sodium citrate.

Useful homologs and fragments thereof that do not
20 occur naturally are designed using known methods for identifying regions of an antigen that are likely to tolerate amino acid sequence changes and/or deletions. As an example, homologous polypeptides from different species are compared; conserved sequences are identified. The more divergent
25 sequences are the most likely to tolerate sequence changes. Homology among sequences may be analyzed using, as an example, the BLAST homology searching algorithm of Altschul et al., Nucleic Acids Res.; 25:3389-3402 (1997). Alternatively, sequences are modified such that they become more reactive to
30 T- and/or B-cells, based on computer-assisted analysis of probable T- or B-cell epitopes. Yet another alternative is to mutate a particular amino acid residue or sequence within the polypeptide *in vitro*, then screen the mutant polypeptides for

their ability to prevent or treat Chlamydia infection according to the method outlined below.

A person skilled in the art will readily understand that by following the screening process of this invention, it will be determined without undue experimentation whether a particular homolog of SEQ ID No: 2, 4 or 6 may be useful in the prevention or treatment of Chlamydia infection. The screening procedure comprises the steps:

- (i) immunizing an animal, preferably mouse, with the test homolog or fragment;
- (ii) inoculating the immunized animal with Chlamydia; and
- (iii) selecting those homologs or fragments which confer protection against Chlamydia.

By "conferring protection" is meant that there is a reduction in severity of any of the effects of Chlamydia infection, in comparison with a control animal which was not immunized with the test homolog or fragment.

Consistent with the first aspect of the invention, polypeptide derivatives are provided that are partial sequences of SEQ ID No: 2, 4 or 6, partial sequences of polypeptide sequences homologous to SEQ ID No: 2, 4 or 6, polypeptides derived from full-length polypeptides by internal deletion, and fusion proteins.

It is an accepted practice in the field of immunology to use fragments and variants of protein immunogens as vaccines, as all that is required to induce an immune response to a protein is a small (e.g., 8 to 10 amino acid) immunogenic region of the protein. Various short synthetic peptides corresponding to surface-exposed antigens of pathogens other than *Chlamydia* have been shown to be effective vaccine antigens against their respective pathogens, e.g. an 11 residue peptide of murine mammary tumor virus (Casey & Davidson, Nucl. Acid Res. (1977) 4:1539), a 16-residue peptide of Semliki Forest

virus (Snijders et al., 1991. J. Gen. Virol. 72:557-565), and two overlapping peptides of 15 residues each from canine parvovirus (Langeveld et al., Vaccine 12(15):1473-1480, 1994).

Accordingly, it will be readily apparent to one skilled in the art, having read the present description, that partial sequences of SEQ ID No: 2, 4 or 6 or their homologous amino acid sequences are inherent to the full-length sequences and are taught by the present invention. Such polypeptide fragments preferably are at least 12 amino acids in length. Advantageously, they are at least 20 amino acids, preferably at least 50 amino acids, and more preferably at least 75 amino acids and most preferably at least 100 amino acids in length.

Polynucleotides of 30 to 600 nucleotides encoding partial sequences of sequences homologous to SEQ ID No: 2, 4 or 6 are retrieved by PCR amplification using the parameters outlined above and using primers matching the sequences upstream and downstream of the 5' and 3' ends of the fragment to be amplified. The template polynucleotide for such amplification is either the full length polynucleotide homologous to SEQ ID No: 1, 3 or 5, or a polynucleotide contained in a mixture of polynucleotides such as a DNA or RNA library. As an alternative method for retrieving the partial sequences, screening hybridization is carried out under conditions described above and using the formula for calculating T_m . Where fragments of 30 to 600 nucleotides are to be retrieved, the calculated T_m is corrected by subtracting $(600/\text{polynucleotide size in base pairs})$ and the stringency conditions are defined by a hybridization temperature that is 5 to 10°C below T_m . Where oligonucleotides shorter than 20-30 bases are to be obtained, the formula for calculating the T_m is as follows: $T_m = 4 \times (G+C) + 2 (A+T)$. For example, an 18 nucleotide fragment of 50% G+C would have an approximate T_m of 54°C. Short peptides that are fragments of SEQ ID No: 2, 4 or 6 or its homologous sequences, are obtained directly by

chemical synthesis (E. Gross and H. J. Meinhofer, 4 The Peptides: Analysis, Synthesis, Biology; Modern Techniques of Peptide Synthesis, John Wiley & Sons (1981), and M. Bodanzki, Principles of Peptide Synthesis, Springer-Verlag (1984)).

5 Useful polypeptide derivatives, e.g., polypeptide fragments, are designed using computer-assisted analysis of amino acid sequences. This would identify probable surface-exposed, antigenic regions (Hughes et al., 1992. Infect. Immun. 60(9):3497). Analysis of 6 amino acid sequences contained in
10 SEQ ID No: 2, 4 or 6, based on the product of flexibility and hydrophobicity propensities using the program SEQSEE (Wishart DS, et al. "SEQSEE: a comprehensive program suite for protein sequence analysis." *Comput Appl Biosci.* 1994 Apr;10(2):121-32), can reveal potential B- and T-cell epitopes which may be used
15 as a basis for selecting useful immunogenic fragments and variants. This analysis uses a reasonable combination of external surface features that is likely to be recognized by antibodies. Probable T-cell epitopes for HLA-A0201 MHC subclass may be revealed by an algorithms that emulate an
20 approach developed at the NIH (Parker KC, et al. "Peptide binding to MHC class I molecules: implications for antigenic peptide prediction." *Immunol Res* 1995;14(1):34-57).

Epitopes which induce a protective T cell-dependent immune response are present throughout the length of the
25 polypeptide. However, some epitopes may be masked by secondary and tertiary structures of the polypeptide. To reveal such masked epitopes large internal deletions are created which remove much of the original protein structure and exposes the masked epitopes. Such internal deletions sometimes effect the
30 additional advantage of removing immunodominant regions of high variability among strains.

Polynucleotides encoding polypeptide fragments and polypeptides having large internal deletions are constructed using standard methods (Ausubel et al., Current Protocols in

Molecular Biology, John Wiley & Sons Inc., 1994). Such methods include standard PCR, inverse PCR, restriction enzyme treatment of cloned DNA molecules, or the method of Kunkel *et al.*

(Kunkel *et al.* Proc. Natl. Acad. Sci. USA (1985) 82:448).

- 5 Components for these methods and instructions for their use are readily available from various commercial sources such as Stratagene. Once the deletion mutants have been constructed, they are tested for their ability to prevent or treat Chlamydia infection as described above.

- 10 As used herein, a fusion polypeptide is one that contains a polypeptide or a polypeptide derivative of the invention fused at the N- or C-terminal end to any other polypeptide (hereinafter referred to as a peptide tail). A simple way to obtain such a fusion polypeptide is by
- 15 translation of an in-frame fusion of the polynucleotide sequences, *i.e.*, a hybrid gene. The hybrid gene encoding the fusion polypeptide is inserted into an expression vector which is used to transform or transfect a host cell. Alternatively, the polynucleotide sequence encoding the polypeptide or
- 20 polypeptide derivative is inserted into an expression vector in which the polynucleotide encoding the peptide tail is already present. Such vectors and instructions for their use are commercially available, *e.g.* the pMal-c2 or pMal-p2 system from New England Biolabs, in which the peptide tail is a maltose
- 25 binding protein, the glutathione-S-transferase system of Pharmacia, or the His-Tag system available from Novagen. These and other expression systems provide convenient means for further purification of polypeptides and derivatives of the invention.

- 30 An advantageous example of a fusion polypeptide is one where the polypeptide or homolog or fragment of the invention is fused to a polypeptide having adjuvant activity, such as subunit B of either cholera toxin or *E. coli* heat-labile toxin. Another advantageous fusion is one where the

polypeptide, homolog or fragment is fused to a strong T-cell epitope or B-cell epitope. Such an epitope may be one known in the art (e.g. the Hepatitis B virus core antigen, D.R. Millich et al., "Antibody production to the nucleocapsid and envelope of the Hepatitis B virus primed by a single synthetic T cell site", Nature. 1987. 329:547-549), or one which has been identified in another polypeptide of the invention based on computer-assisted analysis of probable T- or B-cell epitopes. Consistent with this aspect of the invention is a fusion polypeptide comprising T- or B-cell epitopes from SEQ ID No: 2, 4 or 6 or its homolog or fragment, wherein the epitopes are derived from multiple variants of said polypeptide or homolog or fragment, each variant differing from another in the location and sequence of its epitope within the polypeptide. Such a fusion is effective in the prevention and treatment of Chlamydia infection since it optimizes the T- and B-cell response to the overall polypeptide, homolog or fragment.

To effect fusion, the polypeptide of the invention is fused to the N-, or preferably, to the C-terminal end of the polypeptide having adjuvant activity or T- or B-cell epitope. Alternatively, a polypeptide fragment of the invention is inserted internally within the amino acid sequence of the polypeptide having adjuvant activity. The T- or B-cell epitope may also be inserted internally within the amino acid sequence of the polypeptide of the invention.

Consistent with the first aspect, the polynucleotides of the invention also encode hybrid precursor polypeptides containing heterologous signal peptides, which mature into polypeptides of the invention. By "heterologous signal peptide" is meant a signal peptide that is not found in naturally-occurring precursors of polypeptides of the invention.

Polynucleotide molecules according to the invention, including RNA, DNA, or modifications or combinations thereof,

have various applications. A DNA molecule is used, for example, (i) in a process for producing the encoded polypeptide in a recombinant host system, (ii) in the construction of vaccine vectors such as poxviruses, which are further used in
5 methods and compositions for preventing and/or treating *Chlamydia* infection, (iii) as a vaccine agent (as well as an RNA molecule), in a naked form or formulated with a delivery vehicle and, (iv) in the construction of attenuated *Chlamydia* strains that can over-express a polynucleotide of the invention
10 or express it in a non-toxic, mutated form.

Accordingly, a second aspect of the invention encompasses (i) an expression cassette containing a DNA molecule of the invention placed under the control of the elements required for expression, in particular under the
15 control of an appropriate promoter; (ii) an expression vector containing an expression cassette of the invention; (iii) a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, as well as (iv) a process for producing a polypeptide or polypeptide
20 derivative encoded by a polynucleotide of the invention, which involves culturing a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, under conditions that allow expression of the DNA molecule of the invention and, recovering the encoded
25 polypeptide or polypeptide derivative from the cell culture.

A recombinant expression system is selected from procaryotic and eucaryotic hosts. Eucaryotic hosts include yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris*), mammalian cells (e.g., COS1, NIH3T3, or JEG3 cells),
30 arthropods cells (e.g., *Spodoptera frugiperda* (SF9) cells), and plant cells. A preferred expression system is a procaryotic host such as *E. coli*. Bacterial and eucaryotic cells are available from a number of different sources including commercial sources to those skilled in the art, e.g., the

American Type Culture Collection (ATCC; Rockville, Maryland). Commercial sources of cells used for recombinant protein expression also provide instructions for usage of the cells.

The choice of the expression system depends on the features desired for the expressed polypeptide. For example, it may be useful to produce a polypeptide of the invention in a particular lipidated form or any other form.

One skilled in the art would readily understand that not all vectors and expression control sequences and hosts would be expected to express equally well the polynucleotides of this invention. With the guidelines described below, however, a selection of vectors, expression control sequences and hosts may be made without undue experimentation and without departing from the scope of this invention.

In selecting a vector, the host must be chosen that is compatible with the vector which is to exist and possibly replicate in it. Considerations are made with respect to the vector copy number, the ability to control the copy number, expression of other proteins such as antibiotic resistance. In selecting an expression control sequence, a number of variables are considered. Among the important variable are the relative strength of the sequence (e.g. the ability to drive expression under various conditions), the ability to control the sequence's function, compatibility between the polynucleotide to be expressed and the control sequence (e.g. secondary structures are considered to avoid hairpin structures which prevent efficient transcription). In selecting the host, unicellular hosts are selected which are compatible with the selected vector, tolerant of any possible toxic effects of the expressed product, able to secrete the expressed product efficiently if such is desired, to be able to express the product in the desired conformation, to be easily scaled up, and to which ease of purification of the final product.

The choice of the expression cassette depends on the host system selected as well as the features desired for the expressed polypeptide. Typically, an expression cassette includes a promoter that is functional in the selected host

5 system and can be constitutive or inducible; a ribosome binding site; a start codon (ATG) if necessary; a region encoding a signal peptide, e.g., a lipidation signal peptide; a DNA molecule of the invention; a stop codon; and optionally a 3' terminal region (translation and/or transcription terminator).

10 The signal peptide encoding region is adjacent to the polynucleotide of the invention and placed in proper reading frame. The signal peptide-encoding region is homologous or heterologous to the DNA molecule encoding the mature polypeptide and is compatible with the secretion apparatus of

15 the host used for expression. The open reading frame constituted by the DNA molecule of the invention, solely or together with the signal peptide, is placed under the control of the promoter so that transcription and translation occur in the host system. Promoters and signal peptide encoding regions

20 are widely known and available to those skilled in the art and include, for example, the promoter of *Salmonella typhimurium* (and derivatives) that is inducible by arabinose (promoter araB) and is functional in Gram-negative bacteria such as *E. coli* (as described in U.S. Patent No. 5,028,530 and in Cagnon et al., (Cagnon et al., Protein Engineering (1991) 4(7):843));

25 the promoter of the gene of bacteriophage T7 encoding RNA polymerase, that is functional in a number of *E. coli* strains expressing T7 polymerase (described in U.S. Patent No. 4,952,496); OspA lipidation signal peptide ; and RlpB

30 lipidation signal peptide (Takase et al., J. Bact. (1987) 169:5692).

The expression cassette is typically part of an expression vector, which is selected for its ability to replicate in the chosen expression system. Expression vectors

(e.g., plasmids or viral vectors) can be chosen, for example, from those described in Pouwels et al. (Cloning Vectors: A Laboratory Manual 1985, Supp. 1987). Suitable expression vectors can be purchased from various commercial sources.

5 Methods for transforming/transfecting host cells with expression vectors are well-known in the art and depend on the host system selected as described in Ausubel et al., (Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994).

10 Upon expression, a recombinant polypeptide of the invention (or a polypeptide derivative) is produced and remains in the intracellular compartment, is secreted/excreted in the extracellular medium or in the periplasmic space, or is embedded in the cellular membrane. The polypeptide is
15 recovered in a substantially purified form from the cell extract or from the supernatant after centrifugation of the recombinant cell culture. Typically, the recombinant polypeptide is purified by antibody-based affinity purification or by other well-known methods that can be readily adapted by a
20 person skilled in the art, such as fusion of the polynucleotide encoding the polypeptide or its derivative to a small affinity binding domain. Antibodies useful for purifying by immunoaffinity the polypeptides of the invention are obtained as described below.

25 A polynucleotide of the invention can also be useful as a vaccine. There are two major routes, either using a viral or bacterial host as gene delivery vehicle (live vaccine vector) or administering the gene in a free form, e.g., inserted into a plasmid. Therapeutic or prophylactic efficacy
30 of a polynucleotide of the invention is evaluated as described below.

 Accordingly, a third aspect of the invention provides (i) a vaccine vector such as a poxvirus, containing a DNA molecule of the invention, placed under the control of elements

required for expression; (ii) a composition of matter comprising a vaccine vector of the invention, together with a diluent or carrier; specifically (iii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a vaccine vector of the invention; (iv) a method for inducing an immune response against *Chlamydia* in a mammal (e.g., a human; alternatively, the method can be used in veterinary applications for treating or preventing *Chlamydia* infection of animals, e.g., cats or birds), which involves administering to the mammal an immunogenically effective amount of a vaccine vector of the invention to elicit a protective or therapeutic immune response to *Chlamydia* ; and particularly, (v) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumonia*, *C. pecorum*) infection, which involves administering a prophylactic or therapeutic amount of a vaccine vector of the invention to an infected individual. Additionally, the third aspect of the invention encompasses the use of a vaccine vector of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection.

As used herein, a vaccine vector expresses one or several polypeptides or derivatives of the invention. The vaccine vector may express additionally a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12), that enhances the immune response (adjuvant effect). It is understood that each of the components to be expressed is placed under the control of elements required for expression in a mammalian cell.

Consistent with the third aspect of the invention is a composition comprising several vaccine vectors, each of them capable of expressing a polypeptide or derivative of the invention. A composition may also comprise a vaccine vector capable of expressing an additional *Chlamydia* antigen, or a

subunit, fragment, homolog, mutant, or derivative thereof; optionally together with or a cytokine such as IL-2 or IL-12.

Vaccination methods for treating or preventing infection in a mammal comprises use of a vaccine vector of the invention to be administered by any conventional route, particularly to a mucosal (e.g., ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. Preferred routes depend upon the choice of the vaccine vector. Treatment may be effected in a single dose or repeated at intervals. The appropriate dosage depends on various parameters understood by skilled artisans such as the vaccine vector itself, the route of administration or the condition of the mammal to be vaccinated (weight, age and the like).

Live vaccine vectors available in the art include viral vectors such as adenoviruses and poxviruses as well as bacterial vectors, e.g., *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, Bacille bilié de Calmette-Guérin (BCG), and *Streptococcus*.

An example of an adenovirus vector, as well as a method for constructing an adenovirus vector capable of expressing a DNA molecule of the invention, are described in U.S. Patent No. 4,920,209. Poxvirus vectors include vaccinia and canary pox virus, described in U.S. Patent No. 4,722,848 and U.S. Patent No. 5,364,773, respectively. (Also see, e.g., Tartaglia et al., Virology (1992) 188:217) for a description of a vaccinia virus vector and Taylor et al, Vaccine (1995) 13:539 for a reference of a canary pox.) Poxvirus vectors capable of expressing a polynucleotide of the invention are obtained by homologous recombination as described in Kieny et al., Nature (1984) 312:163 so that the polynucleotide of the invention is inserted in the viral genome under appropriate conditions for expression in mammalian cells. Generally, the dose of vaccine

viral vector, for therapeutic or prophylactic use, can be of from about 1×10^4 to about 1×10^{11} , advantageously from about 1×10^7 to about 1×10^{10} , preferably of from about 1×10^7 to about 1×10^9 plaque-forming units per kilogram. Preferably, viral vectors are administered parenterally; for example, in 3 doses, 4 weeks apart. It is preferable to avoid adding a chemical adjuvant to a composition containing a viral vector of the invention and thereby minimizing the immune response to the viral vector itself.

10 Non-toxicogenic *Vibrio cholerae* mutant strains that are useful as a live oral vaccine are known. Mekalanos et al., Nature (1983) 306:551 and U.S. Patent No. 4,882,278 describe strains which have a substantial amount of the coding sequence of each of the two *ctxA* alleles deleted so that no functional
15 *cholerae* toxin is produced. WO 92/11354 describes a strain in which the *irgA* locus is inactivated by mutation; this mutation can be combined in a single strain with *ctxA* mutations. WO 94/01533 describes a deletion mutant lacking functional *ctxA* and *attRS1* DNA sequences. These mutant strains are genetically
20 engineered to express heterologous antigens, as described in WO 94/19482. An effective vaccine dose of a *Vibrio cholerae* strain capable of expressing a polypeptide or polypeptide derivative encoded by a DNA molecule of the invention contains about 1×10^5 to about 1×10^9 , preferably about 1×10^6 to about
25 1×10^8 , viable bacteria in a volume appropriate for the selected route of administration. Preferred routes of administration include all mucosal routes; most preferably, these vectors are administered intranasally or orally.

Attenuated *Salmonella typhimurium* strains,
30 genetically engineered for recombinant expression of heterologous antigens or not, and their use as oral vaccines are described in Nakayama et al. (Bio/Technology (1988) 6:693) and WO 92/11361. Preferred routes of administration include

all mucosal routes; most preferably, these vectors are administered intranasally or orally.

Other bacterial strains used as vaccine vectors in the context of the present invention are described for *Shigella*
5 *flexneri* in High et al., EMBO (1992) 11:1991 and Sizemore et al., Science (1995) 270:299; for *Streptococcus gordonii* in Medaglini et al., Proc. Natl. Acad. Sci. USA (1995) 92:6868; and for Bacille Calmette Guerin in Flynn J.L., Cell. Mol. Biol. (1994) 40 (suppl. I):31, WO 88/06626, WO 90/00594, WO 91/13157,
10 WO 92/01796, and WO 92/21376.

In bacterial vectors, the polynucleotide of the invention is inserted into the bacterial genome or remains in a free state as part of a plasmid.

The composition comprising a vaccine bacterial vector
15 of the present invention may further contain an adjuvant. A number of adjuvants are known to those skilled in the art. Preferred adjuvants are selected as provided below.

Accordingly, a fourth aspect of the invention provides (i) a composition of matter comprising a
20 polynucleotide of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a polynucleotide of the invention; (iii) a method for inducing an immune response against *Chlamydia* in a mammal by administration
25 of an immunogenically effective amount of a polynucleotide of the invention to elicit a protective immune response to *Chlamydia*; and particularly, (iv) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a
30 prophylactic or therapeutic amount of a polynucleotide of the invention to an infected individual. Additionally, the fourth aspect of the invention encompasses the use of a polynucleotide of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection. A preferred

use includes the use of a DNA molecule placed under conditions for expression in a mammalian cell, especially in a plasmid that is unable to replicate in mammalian cells and to substantially integrate in a mammalian genome.

5 Use of the polynucleotides of the invention include their administration to a mammal as a vaccine, for therapeutic or prophylactic purposes. Such polynucleotides are used in the form of DNA as part of a plasmid that is unable to replicate in a mammalian cell and unable to integrate into the mammalian
10 genome. Typically, such a DNA molecule is placed under the control of a promoter suitable for expression in a mammalian cell. The promoter functions either ubiquitously or tissue-specifically. Examples of non-tissue specific promoters include the early Cytomegalovirus (CMV) promoter (described in
15 U.S. Patent No. 4,168,062) and the Rous Sarcoma Virus promoter (described in Norton & Coffin, *Molec. Cell Biol.* (1985) 5:281). An example of a tissue-specific promoter is the desmin promoter which drives expression in muscle cells (Li et al., *Gene* (1989) 78:243, Li & Paulin, *J. Biol. Chem.* (1991) 266:6562 and Li &
20 Paulin, *J. Biol. Chem.* (1993) 268:10403). Use of promoters is well-known to those skilled in the art. Useful vectors are described in numerous publications, specifically WO 94/21797 and Hartikka et al., *Human Gene Therapy* (1996) 7:1205.

Polynucleotides of the invention which are used as
25 vaccines encode either a precursor or a mature form of the corresponding polypeptide. In the precursor form, the signal peptide is either homologous or heterologous. In the latter case, a eucaryotic leader sequence such as the leader sequence of the tissue-type plasminogen factor (tPA) is preferred.

30 As used herein, a composition of the invention contains one or several polynucleotides with optionally at least one additional polynucleotide encoding another *Chlamydia* antigen such as urease subunit A, B, or both, or a fragment, derivative, mutant, or analog thereof. The composition may

also contain an additional polynucleotide encoding a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12) so that the immune response is enhanced. These additional polynucleotides are placed under appropriate control for
5 expression. Advantageously, DNA molecules of the invention and/or additional DNA molecules to be included in the same composition, are present in the same plasmid.

Standard techniques of molecular biology for preparing and purifying polynucleotides are used in the
10 preparation of polynucleotide therapeutics of the invention. For use as a vaccine, a polynucleotide of the invention is formulated according to various methods outlined below.

One method utilizes the polynucleotide in a naked form, free of any delivery vehicles. Such a polynucleotide is
15 simply diluted in a physiologically acceptable solution such as sterile saline or sterile buffered saline, with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength, such as provided by a sucrose solution, e.g., a
20 solution containing 20% sucrose.

An alternative method utilizes the polynucleotide in association with agents that assist in cellular uptake. Examples of such agents are (i) chemicals that modify cellular permeability, such as bupivacaine (see, e.g., WO 94/16737),
25 (ii) liposomes for encapsulation of the polynucleotide, or (iii) cationic lipids or silica, gold, or tungsten microparticles which associate themselves with the polynucleotides.

Anionic and neutral liposomes are well-known in the
30 art (see, e.g., Liposomes: A Practical Approach, RPC New Ed, IRL press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides.

Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include LipofectinTM also known as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1,2-bis(oleyloxy)-3-
5 (trimethylammonio)propane), DDAB (dimethyldioctadecylammonium bromide), DOGS (dioctadecylamidologlycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702,
10 WO 90/11092, U.S. Patent No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Patent No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleoyl phosphatidylethanolamine), as described in WO 90/11092 as an example.

15 Formulation containing cationic liposomes may optionally contain other transfection-facilitating compounds. A number of them are described in WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/02397. They include spermine derivatives useful for facilitating the transport of DNA through the
20 nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine S, and cationic bile salts (see, for example, WO 93/19768).

Gold or tungsten microparticles are used for gene delivery, as described in WO 91/00359, WO 93/17706, and Tang et
25 al. Nature (1992) 356:152. The microparticle-coated polynucleotide is injected via intradermal or intraepidermal routes using a needleless injection device ("gene gun"), such as those described in U.S. Patent No. 4,945,050, U.S. Patent No. 5,015,580, and WO 94/24263.

30 The amount of DNA to be used in a vaccine recipient depends, e.g., on the strength of the promoter used in the DNA construct, the immunogenicity of the expressed gene product, the condition of the mammal intended for administration (e.g., the weight, age, and general health of the mammal), the mode of

administration, and the type of formulation. In general, a therapeutically or prophylactically effective dose from about 1 μ g to about 1 mg, preferably, from about 10 μ g to about 800 μ g and, more preferably, from about 25 μ g to about 250 μ g, can be administered to human adults. The administration can be achieved in a single dose or repeated at intervals.

The route of administration is any conventional route used in the vaccine field. As general guidance, a polynucleotide of the invention is administered via a mucosal surface, e.g., an ocular, intranasal, pulmonary, oral, intestinal, rectal, vaginal, and urinary tract surface; or via a parenteral route, e.g., by an intravenous, subcutaneous, intraperitoneal, intradermal, intraepidermal, or intramuscular route. The choice of administration route depends on the formulation that is selected. A polynucleotide formulated in association with bupivacaine is advantageously administered into muscles. When a neutral or anionic liposome or a cationic lipid, such as DOTMA or DC-Chol, is used, the formulation can be advantageously injected via intravenous, intranasal (aerosolization), intramuscular, intradermal, and subcutaneous routes. A polynucleotide in a naked form can advantageously be administered via the intramuscular, intradermal, or subcutaneous routes.

Although not absolutely required, such a composition can also contain an adjuvant. If so, a systemic adjuvant that does not require concomitant administration in order to exhibit an adjuvant effect is preferable such as, e.g., QS21, which is described in U.S. Patent No. 5,057,546.

The sequence information provided in the present application enables the design of specific nucleotide probes and primers that are used for diagnostic purposes. Accordingly, a fifth aspect of the invention provides a nucleotide probe or primer having a sequence found in or

derived by degeneracy of the genetic code from a sequence shown in SEQ ID No: 1, 3 or 5

The term "probe" as used in the present application refers to DNA (preferably single stranded) or RNA molecules (or
5 modifications or combinations thereof) that hybridize under the stringent conditions, as defined above, to nucleic acid molecules having SEQ ID No: 1, 3 or 5 or to sequences homologous to SEQ ID No:1, 3 or 5, or to its complementary or anti-sense sequence. Generally, probes are significantly
10 shorter than full-length sequences. Such probes contain from about 5 to about 100, preferably from about 10 to about 80, nucleotides. In particular, probes have sequences that are at least 75%, preferably at least 85%, more preferably 95% homologous to a portion of SEQ ID No:1, 3 or 5 or that are
15 complementary to such sequences. Probes may contain modified bases such as inosine, methyl-5-deoxycytidine, deoxyuridine, dimethylamino-5-deoxyuridine, or diamino-2, 6-purine. Sugar or phosphate residues may also be modified or substituted. For example, a deoxyribose residue may be replaced by a polyamide
20 (Nielsen et al., Science (1991) 254:1497) and phosphate residues may be replaced by ester groups such as diphosphate, alkyl, arylphosphonate and phosphorothioate esters. In addition, the 2'-hydroxyl group on ribonucleotides may be modified by including such groups as alkyl groups.

25 Probes of the invention are used in diagnostic tests, as capture or detection probes. Such capture probes are conventionally immobilized on a solid support, directly or indirectly, by covalent means or by passive adsorption. A detection probe is labeled by a detection marker selected from:
30 radioactive isotopes, enzymes such as peroxidase, alkaline phosphatase, and enzymes able to hydrolyze a chromogenic, fluorogenic, or luminescent substrate, compounds that are chromogenic, fluorogenic, or luminescent, nucleotide base analogs, and biotin.

Probes of the invention are used in any conventional hybridization technique, such as dot blot (Maniatis et al., Molecular Cloning: A Laboratory Manual (1982) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York),
5 Southern blot (Southern, J. Mol. Biol. (1975) 98:503), northern blot (identical to Southern blot with the exception that RNA is used as a target), or the sandwich technique (Dunn et al., Cell (1977) 12:23). The latter technique involves the use of a specific capture probe and/or a specific detection probe with
10 nucleotide sequences that at least partially differ from each other.

A primer is a probe of usually about 10 to about 40 nucleotides that is used to initiate enzymatic polymerization of DNA in an amplification process (e.g., PCR),
15 in an elongation process, or in a reverse transcription method. Primers used in diagnostic methods involving PCR are labeled by methods known in the art.

As described herein, the invention also encompasses
(i) a reagent comprising a probe of the invention for detecting
20 and/or identifying the presence of *Chlamydia* in a biological material; (ii) a method for detecting and/or identifying the presence of *Chlamydia* in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA or RNA is extracted from the material and denatured,
25 and (c) exposed to a probe of the invention, for example, a capture, detection probe or both, under stringent hybridization conditions, such that hybridization is detected; and (iii) a method for detecting and/or identifying the presence of *Chlamydia* in a biological material, in which (a) a sample is
30 recovered or derived from the biological material, (b) DNA is extracted therefrom, (c) the extracted DNA is primed with at least one, and preferably two, primers of the invention and amplified by polymerase chain reaction, and (d) the amplified DNA fragment is produced.

It is apparent that disclosure of polynucleotide sequences of SEQ ID No: 1, 3 or 5, its homologs and partial sequences enable their corresponding amino acid sequences. Accordingly, a sixth aspect of the invention features a
5 substantially purified polypeptide or polypeptide derivative having an amino acid sequence encoded by a polynucleotide of the invention.

A "substantially purified polypeptide" as used herein is defined as a polypeptide that is separated from the
10 environment in which it naturally occurs and/or that is free of the majority of the polypeptides that are present in the environment in which it was synthesized. For example, a substantially purified polypeptide is free from cytoplasmic polypeptides. Those skilled in the art would readily
15 understand that the polypeptides of the invention may be purified from a natural source, *i.e.*, a *Chlamydia* strain, or produced by recombinant means.

Consistent with the sixth aspect of the invention are polypeptides, homologs or fragments which are modified or
20 treated to enhance their immunogenicity in the target animal, in whom the polypeptide, homolog or fragments are intended to confer protection against *Chlamydia*. Such modifications or treatments include: amino acid substitutions with an amino acid derivative such as 3-methylhistidine, 4-hydroxyproline, 5-
25 hydroxylysine etc., modifications or deletions which are carried out after preparation of the polypeptide, homolog or fragment, such as the modification of free amino, carboxyl or hydroxyl side groups of the amino acids.

Identification of homologous polypeptides or
30 polypeptide derivatives encoded by polynucleotides of the invention which have specific antigenicity is achieved by screening for cross-reactivity with an antiserum raised against the polypeptide of reference having an amino acid sequence of SEQ ID No: 1, 3 or 5. The procedure is as follows: a

monospecific hyperimmune antiserum is raised against a purified reference polypeptide, a fusion polypeptide (for example, an expression product of MBP, GST, or His-tag systems, the description and instructions for use of which are contained in
5 Invitrogen product manuals for pcDNA3.1/Myc-His(+) A, B, and C and for the Xpress[™] System Protein Purification), or a synthetic peptide predicted to be antigenic. Where an antiserum is raised against a fusion polypeptide, two different fusion systems are employed. Specific antigenicity can be
10 determined according to a number of methods, including Western blot (Towbin et al., Proc. Natl. Acad. Sci. USA (1979) 76:4350), dot blot, and ELISA, as described below.

In a Western blot assay, the product to be screened, either as a purified preparation or a total *E. coli* extract, is
15 submitted to SDS-Page electrophoresis as described by Laemmli (Nature (1970) 227:680). After transfer to a nitrocellulose membrane, the material is further incubated with the monospecific hyperimmune antiserum diluted in the range of dilutions from about 1:5 to about 1:5000, preferably from about
20 1:100 to about 1:500. Specific antigenicity is shown once a band corresponding to the product exhibits reactivity at any of the dilutions in the above range.

In an ELISA assay, the product to be screened is preferably used as the coating antigen. A purified preparation
25 is preferred, although a whole cell extract can also be used. Briefly, about 100 μ l of a preparation at about 10 μ g protein/ml are distributed into wells of a 96-well polycarbonate ELISA plate. The plate is incubated for 2 hours at 37°C then overnight at 4°C. The plate is washed with
30 phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBS/Tween buffer). The wells are saturated with 250 μ l PBS containing 1% bovine serum albumin (BSA) to prevent non-specific antibody binding. After 1 hour incubation at 37°C, the plate is washed with PBS/Tween buffer. The antiserum is

serially diluted in PBS/Tween buffer containing 0.5% BSA. 100 μ l of dilutions are added per well. The plate is incubated for 90 minutes at 37°C, washed and evaluated according to standard procedures. For example, a goat anti-rabbit peroxidase conjugate is added to the wells when specific antibodies were raised in rabbits. Incubation is carried out for 90 minutes at 37°C and the plate is washed. The reaction is developed with the appropriate substrate and the reaction is measured by colorimetry (absorbance measured spectrophotometrically).

Under the above experimental conditions, a positive reaction is shown by O.D. values greater than a non immune control serum.

In a dot blot assay, a purified product is preferred, although a whole cell extract can also be used. Briefly, a solution of the product at about 100 μ g/ml is serially two-fold diluted in 50 mM Tris-HCl (pH 7.5). 100 μ l of each dilution are applied to a nitrocellulose membrane 0.45 μ m set in a 96-well dot blot apparatus (Biorad). The buffer is removed by applying vacuum to the system. Wells are washed by addition of 50 mM Tris-HCl (pH 7.5) and the membrane is air-dried. The membrane is saturated in blocking buffer (50 mM Tris-HCl (pH 7.5) 0.15 M NaCl, 10 g/L skim milk) and incubated with an antiserum dilution from about 1:50 to about 1:5000, preferably about 1:500. The reaction is revealed according to standard procedures. For example, a goat anti-rabbit peroxidase conjugate is added to the wells when rabbit antibodies are used. Incubation is carried out 90 minutes at 37°C and the blot is washed. The reaction is developed with the appropriate substrate and stopped. The reaction is measured visually by the appearance of a colored spot, e.g., by colorimetry. Under the above experimental conditions, a positive reaction is shown once a colored spot is associated with a dilution of at least about 1:5, preferably of at least about 1:500.

Therapeutic or prophylactic efficacy of a polypeptide or derivative of the invention can be evaluated as described

below. A seventh aspect of the invention provides (i) a composition of matter comprising a polypeptide of the invention together with a diluent or carrier; specifically (ii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a polypeptide of the invention; (iii) a method for inducing an immune response against *Chlamydia* in a mammal, by administering to the mammal an immunogenically effective amount of a polypeptide of the invention to elicit a protective immune response to *Chlamydia*; and particularly, (iv) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to an infected individual. Additionally, the seventh aspect of the invention encompasses the use of a polypeptide of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection.

As used herein, the immunogenic compositions of the invention are administered by conventional routes known the vaccine field, in particular to a mucosal (e.g., ocular, intranasal, pulmonary, oral, gastric, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. The choice of administration route depends upon a number of parameters, such as the adjuvant associated with the polypeptide. If a mucosal adjuvant is used, the intranasal or oral route is preferred. If a lipid formulation or an aluminum compound is used, the parenteral route is preferred with the sub-cutaneous or intramuscular route being most preferred. The choice also depends upon the nature of the vaccine agent. For example, a polypeptide of the invention fused to CTB or LTB is best administered to a mucosal surface.

As used herein, the composition of the invention contains one or several polypeptides or derivatives of the invention. The composition optionally contains at least one additional *Chlamydia* antigen, or a subunit, fragment, homolog,
5 mutant, or derivative thereof.

For use in a composition of the invention, a polypeptide or derivative thereof is formulated into or with liposomes, preferably neutral or anionic liposomes, microspheres, ISCOMS, or virus-like-particles (VLPs) to
10 facilitate delivery and/or enhance the immune response. These compounds are readily available to one skilled in the art; for example, see Liposomes: A Practical Approach, RCP New Ed, IRL press (1990).

Adjuvants other than liposomes and the like are also
15 used and are known in the art. Adjuvants may protect the antigen from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. An appropriate
20 selection can conventionally be made by those skilled in the art, for example, from those described below (under the eleventh aspect of the invention).

Treatment is achieved in a single dose or repeated as necessary at intervals, as can be determined readily by one
25 skilled in the art. For example, a priming dose is followed by three booster doses at weekly or monthly intervals. An appropriate dose depends on various parameters including the recipient (e.g., adult or infant), the particular vaccine antigen, the route and frequency of administration, the
30 presence/absence or type of adjuvant, and the desired effect (e.g., protection and/or treatment), as can be determined by one skilled in the art. In general, a vaccine antigen of the invention is administered by a mucosal route in an amount from about 10 μ g to about 500 mg, preferably from about 1 mg to

about 200 mg. For the parenteral route of administration, the dose usually does not exceed about 1 mg, preferably about 100 μ g.

When used as vaccine agents, polynucleotides and
5 polypeptides of the invention may be used sequentially as part of a multistep immunization process. For example, a mammal is initially primed with a vaccine vector of the invention such as a pox virus, e.g., via the parenteral route, and then boosted twice with the polypeptide encoded by the vaccine vector, e.g.,
10 via the mucosal route. In another example, liposomes associated with a polypeptide or derivative of the invention is also used for priming, with boosting being carried out mucosally using a soluble polypeptide or derivative of the invention in combination with a mucosal adjuvant (e.g., LT).

15 A polypeptide derivative of the invention is also used in accordance with the seventh aspect as a diagnostic reagent for detecting the presence of anti-*Chlamydia* antibodies, e.g., in a blood sample. Such polypeptides are about 5 to about 80, preferably about 10 to about 50 amino
20 acids in length. They are either labeled or unlabeled, depending upon the diagnostic method. Diagnostic methods involving such a reagent are described below.

Upon expression of a DNA molecule of the invention, a polypeptide or polypeptide derivative is produced and purified
25 using known laboratory techniques. As described above, the polypeptide or polypeptide derivative may be produced as a fusion protein containing a fused tail that facilitates purification. The fusion product is used to immunize a small mammal, e.g., a mouse or a rabbit, in order to raise antibodies
30 against the polypeptide or polypeptide derivative (monospecific antibodies). Accordingly, an eighth aspect of the invention provides a monospecific antibody that binds to a polypeptide or polypeptide derivative of the invention.

By "monospecific antibody" is meant an antibody that is capable of reacting with a unique naturally-occurring *Chlamydia* polypeptide. An antibody of the invention is either polyclonal or monoclonal. Monospecific antibodies may be
5 recombinant, e.g., chimeric (e.g., constituted by a variable region of murine origin associated with a human constant region), humanized (a human immunoglobulin constant backbone together with hypervariable region of animal, e.g., murine, origin), and/or single chain. Both polyclonal and monospecific
10 antibodies may also be in the form of immunoglobulin fragments, e.g., F(ab)'₂ or Fab fragments. The antibodies of the invention are of any isotype, e.g., IgG or IgA, and polyclonal antibodies are of a single isotype or a mixture of isotypes.

Antibodies against the polypeptides, homologs or
15 fragments of the present invention are generated by immunization of a mammal with a composition comprising said polypeptide, homolog or fragment. Such antibodies may be polyclonal or monoclonal. Methods to produce polyclonal or monoclonal antibodies are well known in the art. For a review,
20 see "Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Eds. E. Harlow and D. Lane (1988), and D.E. Yelton et al., 1981. Ann. Rev. Biochem. 50:657-680. For monoclonal antibodies, see Kohler & Milstein (1975) Nature 256:495-497.

The antibodies of the invention, which are raised to
25 a polypeptide or polypeptide derivative of the invention, are produced and identified using standard immunological assays, e.g., Western blot analysis, dot blot assay, or ELISA (see, e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, NY). The antibodies are
30 used in diagnostic methods to detect the presence of a *Chlamydia* antigen in a sample, such as a biological sample. The antibodies are also used in affinity chromatography for purifying a polypeptide or polypeptide derivative of the invention. As is discussed further below, such antibodies may

be used in prophylactic and therapeutic passive immunization methods.

Accordingly, a ninth aspect of the invention provides (i) a reagent for detecting the presence of *Chlamydia* in a biological sample that contains an antibody, polypeptide, or polypeptide derivative of the invention; and (ii) a diagnostic method for detecting the presence of *Chlamydia* in a biological sample, by contacting the biological sample with an antibody, a polypeptide, or a polypeptide derivative of the invention, such that an immune complex is formed, and by detecting such complex to indicate the presence of *Chlamydia* in the sample or the organism from which the sample is derived.

Those skilled in the art will readily understand that the immune complex is formed between a component of the sample and the antibody, polypeptide, or polypeptide derivative, whichever is used, and that any unbound material is removed prior to detecting the complex. It is understood that a polypeptide reagent is useful for detecting the presence of anti-*Chlamydia* antibodies in a sample, e.g., a blood sample, while an antibody of the invention is used for screening a sample, such as a gastric extract or biopsy, for the presence of *Chlamydia* polypeptides.

For diagnostic applications, the reagent (i.e., the antibody, polypeptide, or polypeptide derivative of the invention) is either in a free state or immobilized on a solid support, such as a tube, a bead, or any other conventional support used in the field. Immobilization is achieved using direct or indirect means. Direct means include passive adsorption (non-covalent binding) or covalent binding between the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with a reagent is first attached to the solid support. For example, if a polypeptide reagent is used, an antibody that binds to it can serve as an anti-reagent, provided that it binds to an epitope that is not

involved in the recognition of antibodies in biological samples. Indirect means may also employ a ligand-receptor system, for example, where a molecule such as a vitamin is grafted onto the polypeptide reagent and the corresponding
5 receptor immobilized on the solid phase. This is illustrated by the biotin-streptavidin system. Alternatively, a peptide tail is added chemically or by genetic engineering to the reagent and the grafted or fused product immobilized by passive adsorption or covalent linkage of the peptide tail.

10 Such diagnostic agents may be included in a kit which also comprises instructions for use. The reagent is labeled with a detection means which allows for the detection of the reagent when it is bound to its target. The detection means may be a fluorescent agent such as fluorescein isocyanate or
15 fluorescein isothiocyanate, or an enzyme such as horse radish peroxidase or luciferase or alkaline phosphatase, or a radioactive element such as ^{125}I or ^{51}Cr .

Accordingly, a tenth aspect of the invention provides a process for purifying, from a biological sample, a
20 polypeptide or polypeptide derivative of the invention, which involves carrying out antibody-based affinity chromatography with the biological sample, wherein the antibody is a monospecific antibody of the invention.

For use in a purification process of the invention,
25 the antibody is either polyclonal or monospecific, and preferably is of the IgG type. Purified IgGs is prepared from an antiserum using standard methods (see, e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, NY.). Conventional chromatography supports, as well
30 as standard methods for grafting antibodies, are described in, e.g., Antibodies: A Laboratory Manual, D. Lane, E. Harlow, Eds. (1988) and outlined below.

Briefly, a biological sample, such as an *C. pneumoniae* extract preferably in a buffer solution, is applied

to a chromatography material, preferably equilibrated with the buffer used to dilute the biological sample so that the polypeptide or polypeptide derivative of the invention (i.e., the antigen) is allowed to adsorb onto the material. The chromatography material, such as a gel or a resin coupled to an antibody of the invention, is in either a batch form or a column. The unbound components are washed off and the antigen is then eluted with an appropriate elution buffer, such as a glycine buffer or a buffer containing a chaotropic agent, e.g., guanidine HCl, or high salt concentration (e.g., 3 M MgCl₂). Eluted fractions are recovered and the presence of the antigen is detected, e.g., by measuring the absorbance at 280 nm.

An eleventh aspect of the invention provides (i) a composition of matter comprising a monospecific antibody of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a monospecific antibody of the invention, and (iii) a method for treating or preventing a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae* or *C. pecorum*) infection, by administering a therapeutic or prophylactic amount of a monospecific antibody of the invention to an infected individual. Additionally, the eleventh aspect of the invention encompasses the use of a monospecific antibody of the invention in the preparation of a medicament for treating or preventing *Chlamydia* infection.

The monospecific antibody is either polyclonal or monoclonal, preferably of the IgA isotype (predominantly). In passive immunization, the antibody is administered to a mucosal surface of a mammal, e.g., the gastric mucosa, e.g., orally or intragastrically, advantageously, in the presence of a bicarbonate buffer. Alternatively, systemic administration, not requiring a bicarbonate buffer, is carried out. A monospecific antibody of the invention is administered as a single active component or as a mixture with at least one

monospecific antibody specific for a different *Chlamydia* polypeptide. The amount of antibody and the particular regimen used are readily determined by one skilled in the art. For example, daily administration of about 100 to 1,000 mg of
5 antibodies over one week, or three doses per day of about 100 to 1,000 mg of antibodies over two or three days, are effective regimens for most purposes.

Therapeutic or prophylactic efficacy are evaluated using standard methods in the art, e.g., by measuring induction
10 of a mucosal immune response or induction of protective and/or therapeutic immunity, using, e.g., the *C. pneumoniae* mouse model. Those skilled in the art will readily recognize that the *C. pneumoniae* strain of the model may be replaced with another *Chlamydia* strain. For example, the efficacy of DNA
15 molecules and polypeptides from *C. pneumoniae* is preferably evaluated in a mouse model using *C. pneumoniae* strain. Protection is determined by comparing the degree of *Chlamydia* infection to that of a control group. Protection is shown when infection is reduced by comparison to the control group. Such
20 an evaluation is made for polynucleotides, vaccine vectors, polypeptides and derivatives thereof, as well as antibodies of the invention.

Adjuvants useful in any of the vaccine compositions described above are as follows.

25 Adjuvants for parenteral administration include aluminum compounds, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate. The antigen is precipitated with, or adsorbed onto, the aluminum compound according to standard protocols. Other adjuvants, such as RIBI
30 (ImmunoChem, Hamilton, MT), are used in parenteral administration.

Adjuvants for mucosal administration include bacterial toxins, e.g., the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostridium difficile* toxin A and

the *pertussis* toxin (PT), or combinations, subunits, toxoids, or mutants thereof such as a purified preparation of native cholera toxin subunit B (CTB). Fragments, homologs, derivatives, and fusions to any of these toxins are also
5 suitable, provided that they retain adjuvant activity. Preferably, a mutant having reduced toxicity is used. Suitable mutants are described, e.g., in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/06627 (Arg-192-Gly LT mutant), and WO 95/34323 (Arg-9-Lys and Glu-129-Gly PT mutant). Additional LT mutants
10 that are used in the methods and compositions of the invention include, e.g., Ser-63-Lys, Ala-69Gly, Glu-110-Asp, and Glu-112-Asp mutants. Other adjuvants, such as a bacterial monophosphoryl lipid A (MPLA) of, e.g., *E. coli*, *Salmonella minnesota*, *Salmonella typhimurium*, or *Shigella flexneri*;
15 saponins, or polylactide glycolide (PLGA) microspheres, is also be used in mucosal administration.

Adjuvants useful for both mucosal and parenteral administrations include polyphosphazene (WO 95/02415), DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol;
20 U.S. Patent No. 5,283,185 and WO 96/14831) and QS-21 (WO 88/09336).

Any pharmaceutical composition of the invention containing a polynucleotide, a polypeptide, a polypeptide derivative, or an antibody of the invention, is manufactured in
25 a conventional manner. In particular, it is formulated with a pharmaceutically acceptable diluent or carrier, e.g., water or a saline solution such as phosphate buffer saline. In general, a diluent or carrier is selected on the basis of the mode and route of administration, and standard pharmaceutical practice.
30 Suitable pharmaceutical carriers or diluents, as well as pharmaceutical necessities for their use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field and in the USP/NF.

The invention also includes methods in which *Chlamydia* infection are treated by oral administration of a *Chlamydia* polypeptide of the invention and a mucosal adjuvant, in combination with an antibiotic, an antacid, sucralfate, or a combination thereof. Examples of such compounds that can be administered with the vaccine antigen and the adjuvant are antibiotics, including, e.g., macrolides, tetracyclines, and derivatives thereof (specific examples of antibiotics that can be used include azithromycin or doxycyclin or immunomodulators such as cytokines or steroids). In addition, compounds containing more than one of the above-listed components coupled together, are used. The invention also includes compositions for carrying out these methods, i.e., compositions containing a *Chlamydia* antigen (or antigens) of the invention, an adjuvant, and one or more of the above-listed compounds, in a pharmaceutically acceptable carrier or diluent.

It has recently been shown that the 9kDa cysteine rich membrane protein contains a sequence cross-reactive with the murine alpha-myosin heavy chain epitope M7A-alpha, an epitope conserved in humans (Bachmaier et al., Science (1999) 283:1335). This cross-reactivity is proposed to contribute to the development of cardiovascular disease, so it may be beneficial to remove this epitope, and any other epitopes cross-reactive with human antigens, from the protein if it is to be used as a vaccine. Accordingly, a further embodiment of the present invention includes the modification of the coding sequence, for example, by deletion or substitution of the nucleotides encoding the epitope from polynucleotides encoding the protein, as to improve the efficacy and safety of the protein as a vaccine. A similar approach may be appropriate for any protective antigen found to have unwanted homologies or cross-reactivities with human antigens.

Amounts of the above-listed compounds used in the methods and compositions of the invention are readily

determined by one skilled in the art. Treatment/immunization schedules are also known and readily designed by one skilled in the art. For example, the non-vaccine components can be administered on days 1-14, and the vaccine antigen + adjuvant can be administered on days 7, 14, 21, and 28.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

20 Example 1:

This example illustrates the preparation of a plasmid vector pCACPNM555a containing the full length 76kDa protein gene.

25 The full-length 76kDa protein gene was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGGTTAATCCTATTGGTCCAGG 3') (SEQ ID No:9) and a 3' primer (5' GCGCCGGATCCCTTGGAGATAACCAGAATATAGAG 3') (SEQ ID No:10). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation codon and a sequence close to the 5' end of the full-length 76kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 76kDa protein and a Bam HI

restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified
5 using QIAquick™ PCR purification kit (Qiagen) and then digested with Not I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector describe in Example 2 (Fig. 4) with transcription under control of the human CMV promoter.

10 Example 2:

This example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was
15 restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR
20 fragment containing the full-length 76kDa protein gene was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCACPNM555a (Fig 4).

The resulting plasmid, pCACPNM555a, was transferred by electroporation into *E. coli* XL-1 blue (Stratagene) which
25 was grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and
30 comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 3:

This example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C.*

5 *pneumoniae*.

It has been previously demonstrated (Yang et. al., 1993) that mice are susceptible to intranasal infection with different isolates of *C. pneumoniae*. Strain AR-39 (Grayston, 1989) was used in Balb/c mice as a challenge infection model to
10 examine the capacity of Chlamydia gene products delivered as naked DNA to elicit a protective response against a sublethal *C. pneumoniae* lung infection. Protective immunity is defined as an accelerated clearance of pulmonary infection.

Groups of 7 to 9 week old male Balb/c mice (7 to 10
15 per group) were immunized intramuscularly (i.m.) plus intranasally (i.n.) with plasmid DNA containing the coding sequence of *C.pneumoniae* full-length 76kDa protein as described in Examples 1 and 2. Saline or the plasmid vector lacking an inserted Chlamydial gene was given to groups of control
20 animals.

For i.m. immunization alternate left and right quadriceps were injected with 100µg of DNA in 50µl of PBS on three occasions at 0, 3 and 6 weeks. For i.n. immunization, anaesthetized mice aspirated 50µl of PBS containing 50 µg DNA
25 on three occasions at 0, 3 and 6 weeks. At week 8, immunized mice were inoculated i.n. with 5×10^5 IFU of *C. pneumoniae*, strain AR39 in 100µl of SPG buffer to test their ability to limit the growth of a sublethal *C. pneumoniae* challenge.

Lungs were taken from mice at days 5 and 9 post-
30 challenge and immediately homogenised in SPG buffer (7.5% sucrose, 5mM glutamate, 12.5mM phosphate pH7.5). The homogenate was stored frozen at -70°C until assay. Dilutions of the homogenate were assayed for the presence of infectious

Chlamydia by inoculation onto monolayers of susceptible cells. The inoculum was centrifuged onto the cells at 3000rpm for 1 hour, then the cells were incubated for three days at 35°C in the presence of 1µg/ml cycloheximide. After incubation the
5 monolayers were fixed with formalin and methanol then immunoperoxidase stained for the presence of Chlamydial inclusions using convalescent sera from rabbits infected with *C.pneumoniae* and metal-enhanced DAB as a peroxidase substrate.

Figure 7 and Table 1 show that mice immunized i.n.
10 and i.m. with pCACPNM555a had Chlamydial lung titers less than 30,000 IFU/lung (mean 23,550) in 5 of 6 cases at day 9 whereas the range of values for control mice sham immunized with saline were 20,800 to 323,300 IFU/lung (mean 206,375) for (Table 1). DNA immunisation *per se* was not responsible for the observed
15 protective effect since two other plasmid DNA constructs, pCACPNM806 and pCACPNM760, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice. The constructs pCACPNM806 and pCACPNM760 are identical to pCACPNM555a except that the
20 nucleotide sequence encoding the full-length 76kDa protein is replaced with *C. pneumoniae* nucleotide sequences encoding an unrelated sequence.

Table 1

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS			
	IMMUNIZING CONSTRUCT			
	Saline	pCACPNM806	pCACPNM760	pCACPNM555a
	Day 9	Day 9	Day 9	Day 9
1	225900	36700	140300	27300
2	20800	238700	128400	15200
3	286100	52300	88700	34600
4	106700	109600	25600	20500
5	323300	290000	37200	22000
6	144300	298800	5900	21700
7	261700			
8	282200			
MEAN	206375	171016.667	71016.6667	23550
SD	105183.9	119141.32	56306.57	6648.53
Wilcoxon p		0.8518	0.0293	0.008

5 Example 4:

This example illustrates the preparation of a plasmid vector pCAI555 containing a 5'-truncated 76kDa protein gene.

The 5' truncated 76kDa protein gene was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAATGCGGCCGCCACCATGAGTCTGGCAGATAAGCTGGG 3') (SEQ ID No:11) and a 3' primer (5' GCGCCGGATCCCTTGGAGATAACCAGAATATA 3') (SEQ ID No:12). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation codon and a sequence at the second Met codon of the 76kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 3' 76kDa protein and a Bam HI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen) and then digested with Not I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector describe in Example 5 (Fig. 5) with transcription under control of the human CMV promoter.

Example 5:

This example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the 5' truncated 76kDa protein gene was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCAI555 (Fig 5).

The resulting plasmid, pCAI555, was transferred by electroporation into *E. coli* XL-1 blue (Stratagene) which was grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 6:

This Example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C.*

5 *pneumoniae*. The procedures are described in Example 3 above, except that the DNA plasmid used for immunization contains the coding sequence of *C. pneumoniae* 5'-truncated 76kDa protein, as described in Examples 4 and 5.

Figure 8 and Table 2 show that mice immunized i.n.
10 and i.m. with pCAI555 had Chlamydial lung titers less than 13000 IFU/lung (mean 6050) in 6 of 6 cases at day 9 whereas the range of values for control mice sham immunized with saline were 106,100 IFU/lung (mean 39,625) for (Table 2). DNA immunisation *per se* was not responsible for the observed
15 protective effect since two other plasmid DNA constructs, pCAI116 and pCAI178, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice. The constructs pCAI116 and pCAI178 are identical to pCAI555 except that the nucleotide sequence encoding the 5'-
20 truncated 76kDa protein is replaced with a *C.pneumoniae* nucleotide sequence encoding an unprotective sequence and the nucleoside 5'-diphosphate phosphotransferase protein.

Table 2

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS			
	IMMUNIZING CONSTRUCT			
	Saline	pCAI116	pCAI178	pCAI555
	Day 9	Day 9	Day 9	Day 9
1	1700	47700	80600	6100
2	36200	12600	31900	10700
3	106100	28600	30600	500
4	33500	17700	6500	5100
5	70400	77300	53000	1100
6	48700	17600	79500	12800
7	600			
8	19800			
9	29500			
10	100000			
11	15000			
12	56600			
13	60300			
14	88800			
15	30400			
16	69300			
17	47500			
18	96500			
19	30200			
20	84800			
21	3800			
22	65900			
23	33000			
MEAN	49069.57	33583.33	47016.67	6050
SD	32120.48	24832.67	29524.32	4967.80

Example 7:

5

This example illustrates the preparation of a plasmid vector pCAD76kDa containing a 3'-truncated 76kDa protein gene.

The 3'-truncated 76kDa protein gene was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain

reaction (PCR) using a 5' primer (5'
GCTCTAGACCGCCATGACAAAAAACATTATGCTTGGG 3') (SEQ ID No:13) and a
3' primer (5' CGGGATCCATAGAAGTTGCTGCAGCGGG 3') (SEQ ID No:14).
The 5' primer contains a Xba I restriction site, a ribosome
5 binding site, an initiation codon and a sequence 765bp upstream
of the 5' end of the 76kDa protein coding sequence. The 3'
primer includes a 21bp the sequence downstream of codon 452 of
the 76kDa protein and a Bam HI restriction site. An additional
nucleotide was inserted to obtain an in-frame fusion with the
10 Histidine tag. Note that inclusion of the 765bp 5' region and
the 21bp 3' regions were inadvertent. These sequences are not
part of the 76kDa protein gene. Nevertheless, immunoprotection
was achieved using this sequence (Example 6).

After amplification, the PCR fragment was purified
15 using QIAquick™ PCR purification kit (Qiagen) and then digested
with Xba I and Bam HI and cloned into the pCA-Myc-His
eukaryotic expression vector describe in Example 8 (Fig. 6)
with transcription under control of the human CMV promoter.

20 Example 8:

This Example illustrates the preparation of the
eukaryotic expression vector pCA/Myc-His.

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was
25 restricted with Spe I and Bam HI to remove the CMV promoter and
the remaining vector fragment was isolated. The CMV promoter
and intron A from plasmid VR-1012 (Vical) was isolated on a Spe
I / Bam HI fragment. The fragments were ligated together to
produce plasmid pCA/Myc-His. The Xba I/Bam HI restricted PCR
30 fragment containing a 3'-truncated 76kDa protein gene was
ligated into the Xba I and Bam HI restricted plasmid pCA/Myc-
His to produce plasmid pCAD76kDa (Fig. 6).

The resulting plasmid, pCAD76kDa, was transferred by
electroporation into *E. coli* XL-1 blue (Stratagene) which was

grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified
5 after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

10 Example 9:

This example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*. The procedures are as described in Example 3
15 above, except that the DNA plasmid used for immunization contains the coding sequence of *C. pneumoniae* 3'-truncated 76kDa protein, as described in Examples 7 and 8.

Figure 9 and Table 3 show that mice immunized i.n. and i.m. with pCAD76kDa had Chlamydial lung titers less than
20 2400 in 5 of 5 cases whereas the range of values for control mice were 1800-23100 IFU/lung (mean 11811) and 16600-26100 IFU/lung (mean 22100) for sham immunized with saline or immunized with the unmodified vector respectively (Table 2). The lack of protection with the unmodified vector confirms that
25 DNA per se was not responsible for the observed protective effect. This is further supported by the results obtained for one additional plasmid DNA construct, pdagA, that failed to protect, and for which the mean lung titers were similar to those obtained for saline-immunized control mice. The
30 construct pdagA is identical to pCAD76kDa except that the nucleotide sequence encoding the 3'-truncated 76kDa protein is replaced with a *C.pneumoniae* nucleotide sequence encoding the protein dagA.

Table 3

MOUSE	BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS			
	IMMUNIZING CONSTRUCT			
	Saline	Vector	pdagA	pCAD76kDa
1	17700	19900	16000	1700
2	3900	16600	500	2000
3	1800	24300	18500	2300
4	16400	26100	12800	2100
5	11700	23600	6400	600
6	23100			
7	12000			
8	5300			
9	14400			
10	18700			
11	7300			
12	8400			
MEAN	11725	22100	10840	1740
SD	6567.71	3813.79	7344.59	673.05

CLAIMS:

1. A nucleic acid molecule comprising a nucleic acid sequence which encodes a polypeptide selected from any of:
 - (a) SEQ ID No: 2;
 - 5 (b) SEQ ID No. 4;
 - (c) SEQ ID No. 6;
 - (d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and
 - (e) a polypeptide of any one of (a) to (d) which has been
 - 10 modified to improve its immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d).
2. A nucleic acid molecule comprising a nucleic acid sequence selected from any of:
 - 15 (a) SEQ ID No: 1;
 - (b) SEQ ID No: 3;
 - (c) SEQ ID No: 5;
 - (d) a sequence which encodes a polypeptide encoded by any one of SEQ ID Nos: 1, 3 and 5;
 - 20 (e) a sequence comprising at least 38 consecutive nucleotides from any one of the nucleic acid sequences of (a) to (d); and
 - (f) a sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptides
 - 25 encoded by any one of SEQ ID Nos: 1, 3 and 5.
3. A nucleic acid molecule comprising a nucleic acid sequence which is anti-sense to the nucleic acid molecule of claim 1.
4. A nucleic acid molecule comprising a nucleic acid
- 30 sequence which encodes a fusion protein, said fusion protein comprising a polypeptide encoded by a nucleic acid molecule according to claim 1 and an additional polypeptide.

5. The nucleic acid molecule of claim 4 wherein the additional polypeptide is a heterologous signal peptide.
6. The nucleic acid molecule of claim 4 wherein the additional polypeptide has adjuvant activity.
- 5 7. A nucleic acid molecule according to any one of claims 1 to 6, operatively linked to one or more expression control sequences.
8. A vaccine comprising at least one first nucleic acid according to any one of claims 1, 2, and 4 to 7 and a vaccine
10 vector wherein each first nucleic acid is expressed as a polypeptide, the vaccine optionally comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by said first nucleic acid.
- 15 9. The vaccine of claim 8 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.
10. A pharmaceutical composition comprising a nucleic acid according to any one of claims 1 to 7 and a pharmaceutically acceptable carrier.
- 20 11. A pharmaceutical composition comprising a vaccine according to claim 8 or 9 and a pharmaceutically acceptable carrier.
12. A unicellular host transformed with the nucleic acid molecule of claim 7.
- 25 13. A nucleic acid probe of 5 to 100 nucleotides which hybridizes under stringent conditions to the nucleic acid molecule of SEQ ID No: 1, or to a homolog or complementary or anti-sense sequence of said nucleic acid molecule.
14. A primer of 10 to 40 nucleotides which hybridizes
30 under stringent conditions to the nucleic acid molecules of SEQ ID No: 1, or to a homolog or complementary or anti-sense sequence of said nucleic acid molecule.
15. A polypeptide encoded by a nucleic acid sequence according to any one of claims 1, 2 and 4 to 7.

16. A polypeptide comprising an amino acid sequence selected from any of:

- (a) SEQ ID No: 2;
- (b) SEQ ID No: 4;
- 5 (c) SEQ ID No: 6;
- (d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and
- (e) a polypeptide of any one of (a) to (d) which has been modified to improve its immunogenicity, wherein said modified
- 10 polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d).

17. A fusion polypeptide comprising a polypeptide of claim 15 or 16 and an additional polypeptide.

18. The fusion polypeptide of claim 17 wherein the

15 additional polypeptide is a heterologous signal peptide.

19. The fusion protein of claim 17 wherein the additional polypeptide has adjuvant activity.

20. A method for producing a polypeptide of claim 15 or 16, comprising the step of culturing a unicellular host

20 according to claim 12.

21. An antibody against the polypeptide of any one of claims 15 to 19.

22. A vaccine comprising at least one first polypeptide according to any one of claims 15 to 19 and a pharmaceutically

25 acceptable carrier, optionally comprising a second polypeptide which enhances the immune response to the first polypeptide.

23. The vaccine of claim 22 wherein the second polypeptide comprises an additional *Chlamydia* polypeptide.

24. A pharmaceutical composition comprising a polypeptide

30 according to any one of claims 15 to 19 and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising a vaccine according to claim 22 or 23 and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising an antibody according to claim 21 and a pharmaceutically acceptable carrier.

27. A method for preventing or treating *Chlamydia*
5 infection using:

- (a) the nucleic acid of any one of claims 1 to 7;
- (b) the vaccine of any one of claims 8, 9, 22 and 23;
- (c) the pharmaceutical composition of any one of claims
10, 11, 24 to 26;
- 10 (d) the polypeptide of any one of claims 15 to 19; or
- (e) the antibody of claim 21.

28. A method of detecting *Chlamydia* infection comprising the step of assaying a body fluid of a mammal to be tested, with a component selected from any one of:

- 15 (a) the nucleic acid of any one of claims 1 to 7;
- (b) the polypeptide of any one of claims 15 to 19; and
- (c) the antibody of claim 21.

29. A diagnostic kit comprising instructions for use and a component selected from any one of:

- 20 (a) the nucleic acid of any one of claims 1 to 7;
- (b) the polypeptide of any one of claims 15 to 19; and
- (c) the antibody of claim 21.

30. A method for identifying a polypeptide of claims 15 to 19 which induces an immune response effective to prevent or
25 lessen the severity of *Chlamydia* infection in a mammal previously immunized with polypeptide, comprising the steps of:

- (a) immunizing a mouse with the polypeptide; and
- (b) inoculating the immunized mouse with *Chlamydia*;

wherein the polypeptide which prevents or lessens the
30 severity of *Chlamydia* infection in the immunized mouse compared to a non-immunized control mouse is identified.

31. An expression plasmid selected from the group consisting of pCACPNM555a, pCAI555 and pCAD76kDa.

32. A nucleic acid molecule selected from the group consisting of SEQ ID Nos: 1, 3, 5 and 7.
33. A polypeptide selected from the group consisting of SEQ ID Nos: 2, 4, 6 and 8.
- 5 34. An isolated 76kDa protein from *Chlamydia*.
35. An isolated 76kDa protein from *Chlamydia pneumoniae*

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Figure 1: Full-length Sequence of *C. pneumoniae* 76kDa Gene.

```

ataaaatctt taaaaacagg ctcgcattaa ttattagtga gagctttttt tttatttttt 60

ataataaaaac taaaagattt ttattatttt ttgagttttt atg gtt aat cct att 115
                                         Met Val Asn Pro Ile
                                         1           5

ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat ctt 163
Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp Leu
                        10                15                20

tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa gct 211
Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu Ala
                        25                30                35

caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc gat 259
Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr Asp
                        40                45                50

tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc atg 307
Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu Met
                        55                60                65

agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct tct 355
Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser Ser
70                75                80                85

act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct acg 403
Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro Thr
                        90                95                100

cct cct oca ccc acg ttt gat gat tat aag act caa gcg caa aca gct 451
Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr Ala
                        105                110                115

tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct gct 499
Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala Ala
                        120                125                130

ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg gct 547
Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala Ala
                        135                140                145

act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat gcc 595
Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn Ala
150                155                160                165

gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat gct 643
Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr Ala
                        170                175                180

tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc ttc 691
Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe
                        185                190                195

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Figure 1 (continued)

gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa	739
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys	
200 205 210	
gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca ggg	787
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly	
215 220 225	
aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct aca	835
Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr	
230 235 240 245	
gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat ttt	883
Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe	
250 255 260	
gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat aac	931
Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn	
265 270 275	
agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct aag	979
Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys	
280 285 290	
aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att ctt	1027
Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu	
295 300 305	
caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat	1075
Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn	
310 315 320 325	
atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca gtt	1123
Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr Val	
330 335 340	
gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt tcc	1171
Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val Ser	
345 350 355	
atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg tct	1219
Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met Ser	
360 365 370	
ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat tct	1267
Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp Ser	
375 380 385	
caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa gcc	1315
Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys Ala	
390 395 400 405	
gct gga gat gac agt gct gct gca gcg ctg gca gat gct cag aaa gct	1363
Ala Gly Asp Asp Ser Ala Ala Ala Ala Leu Ala Asp Ala Gln Lys Ala	
410 415 420	

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Figure 1 (continued)

tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc aat	1411
Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu Asn	
425 430 435	
gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gtt cct	1459
Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val Pro	
440 445 450	
ccc gct gca gca agt tct ata ggg tca tct gta aaa cag ctt tac aag	1507
Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val Lys Gln Leu Tyr Lys	
455 460 465	
acc tca aaa tct aca ggt tct gat tat aaa aca cag ata tca gca ggt	1555
Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala Gly	
470 475 480 485	
tat gat gct tac aaa tcc atc aat gat gcc tat ggt agg gca cga aat	1603
Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg Asn	
490 495 500	
gat gcg act cgt gat gtg ata aac aat gta agt acc ccc gct ctc aca	1651
Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu Thr	
505 510 515	
cga tcc gtt cct aga gca cga aca gaa gct cga gga cca gaa aaa aca	1699
Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys Thr	
520 525 530	
gat caa gcc ctc gct agg gtg att tct ggc aat agc aga act ctt gga	1747
Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu Gly	
535 540 545	
gat gtc tat agt caa gtt tcg gca cta caa tct gta atg cag atc atc	1795
Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile Ile	
550 555 560 565	
cag tcg aat cct caa gcg aat aat gag gag atc aga caa aag ctt aca	1843
Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu Thr	
570 575 580	
tcg gca gtg aca aag cct cca cag ttt ggc tat cct tat gtg caa ctt	1891
Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln Leu	
585 590 595	
tct aat gac tct aca cag aag ttc ata gct aaa tta gaa agt ttg ttt	1939
Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu Phe	
600 605 610	
gct gaa gga tct agg aca gca gct gaa ata aaa gca ctt tcc ttt gaa	1987
Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe Glu	
615 620 625	
acg aac tcc ttg ttt att cag cag gtg ctg gtc aat atc ggc tct cta	2035
Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser Leu	
630 635 640 645	

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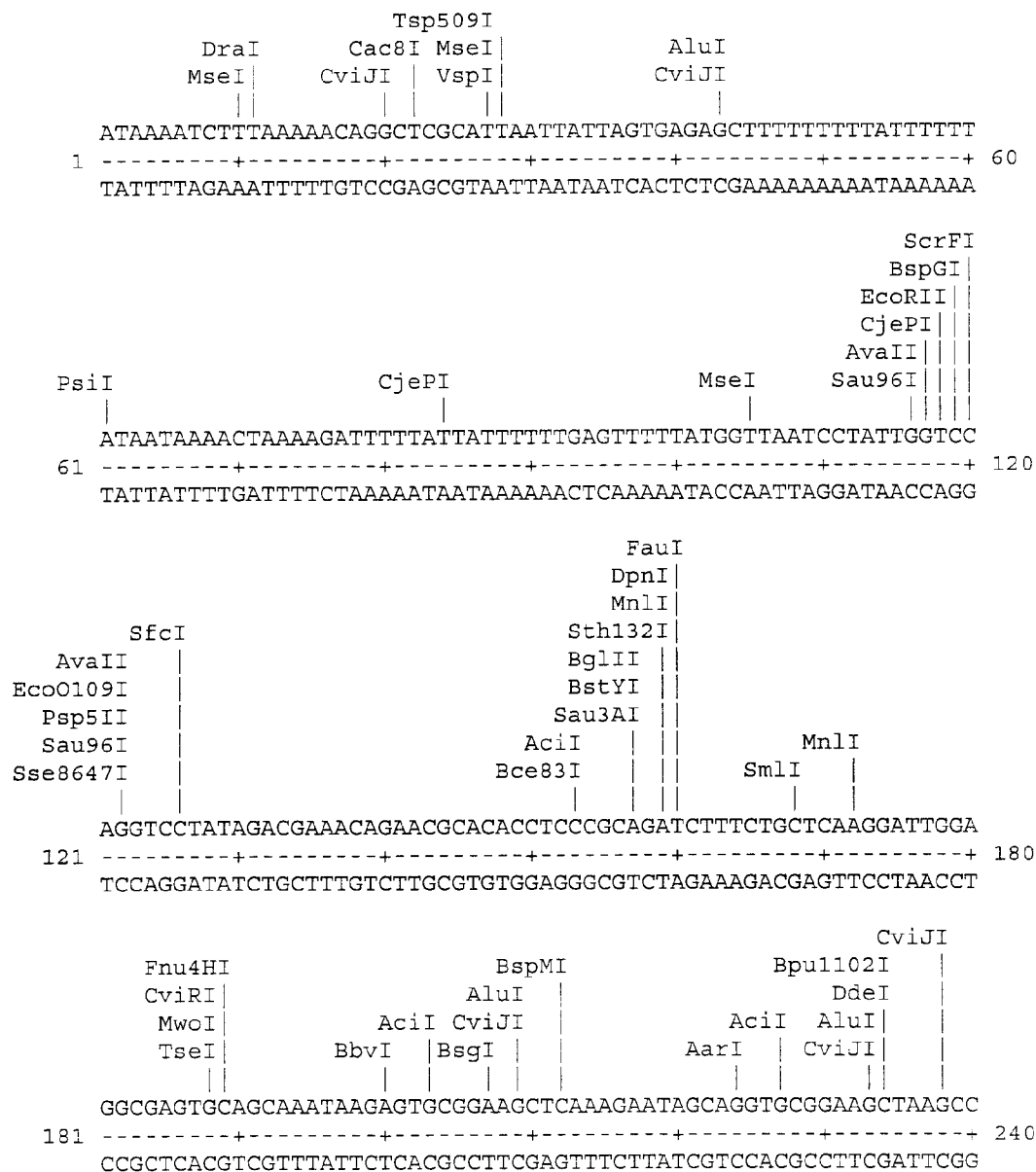
Figure 1 (continued)

tat tct ggt tat ctc caa taacaacacc taagtgttcg tttggagaga 2083
Tyr Ser Gly Tyr Leu Gln
650

ttattatgtg ctttggttaag gcctttgttg aggccttacc aacacactag aacgatcttc 2143

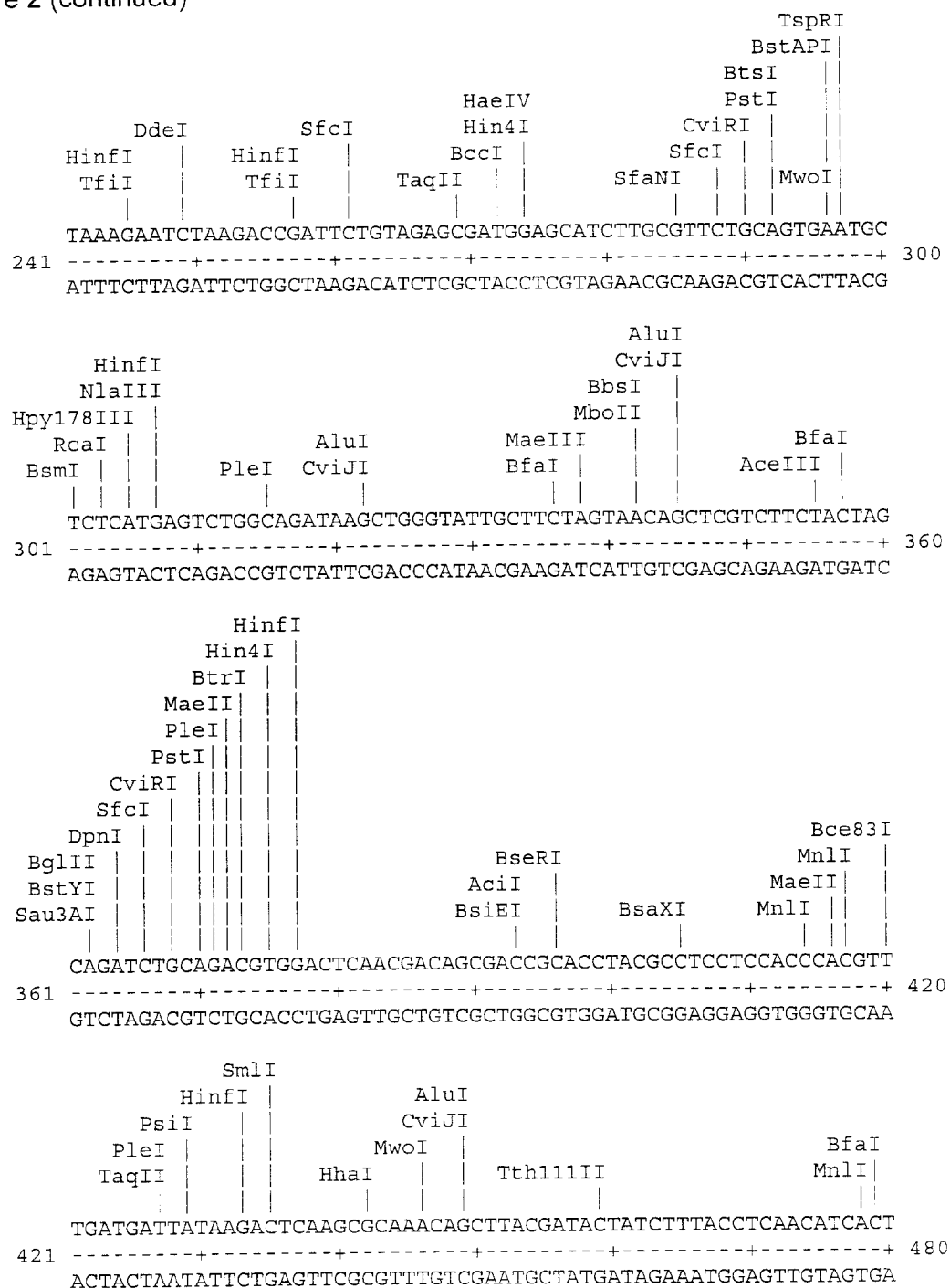
aataaataaa aga 2156

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Figure 2: Restriction enzyme map of *C. pneumoniae* 76kDa gene.

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Figure 2 (continued)



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Figure 2 (continued)

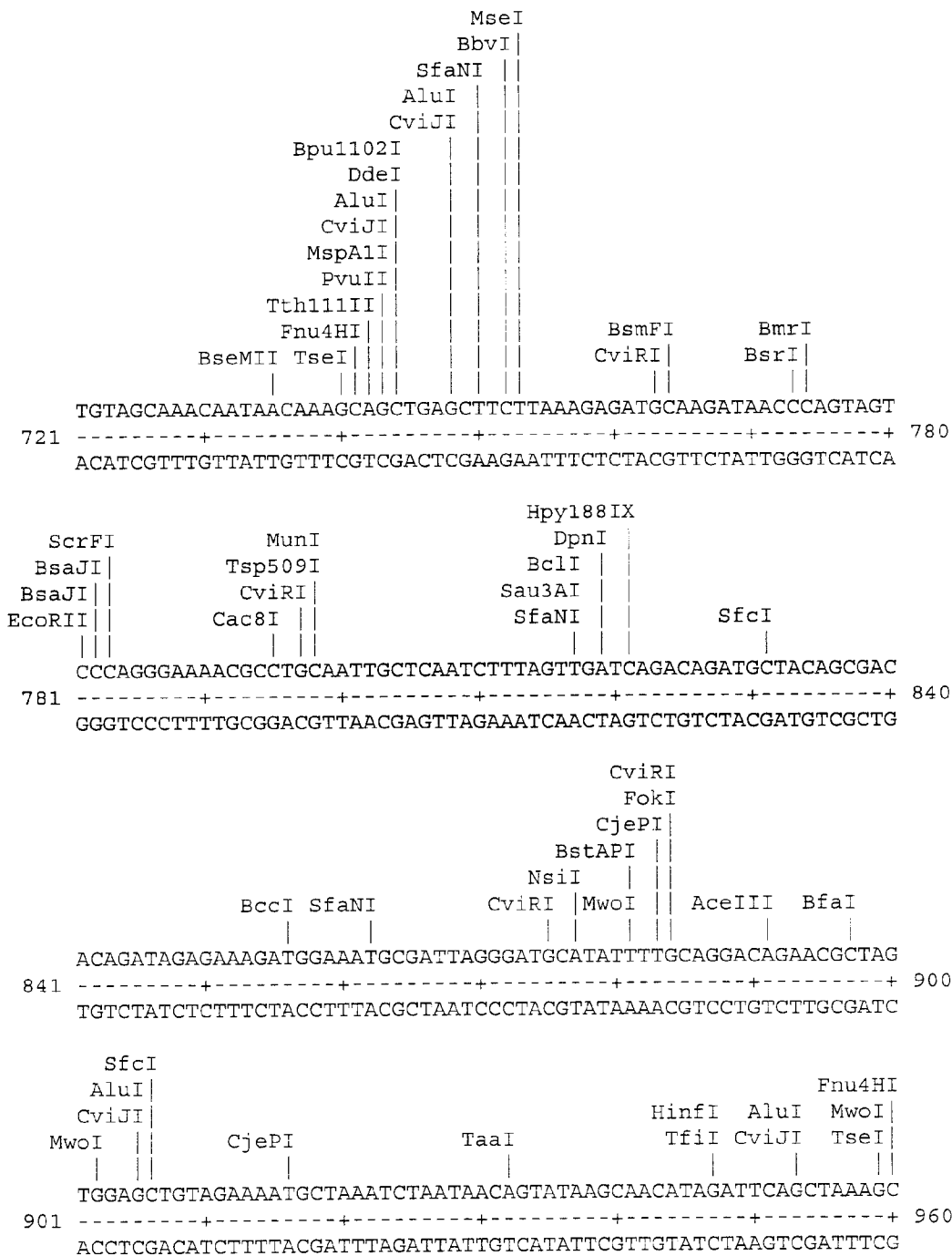


Figure 2 (continued)

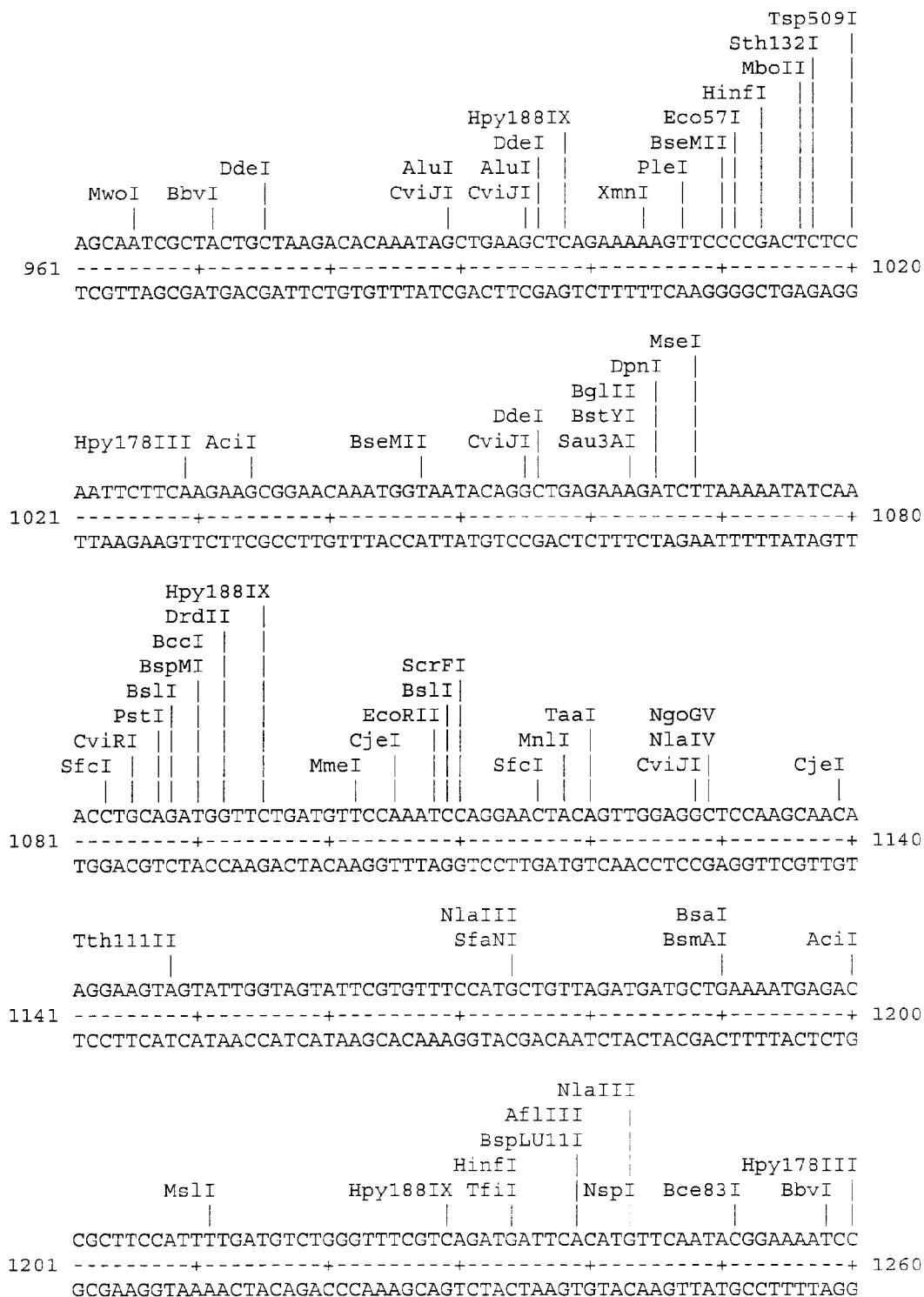
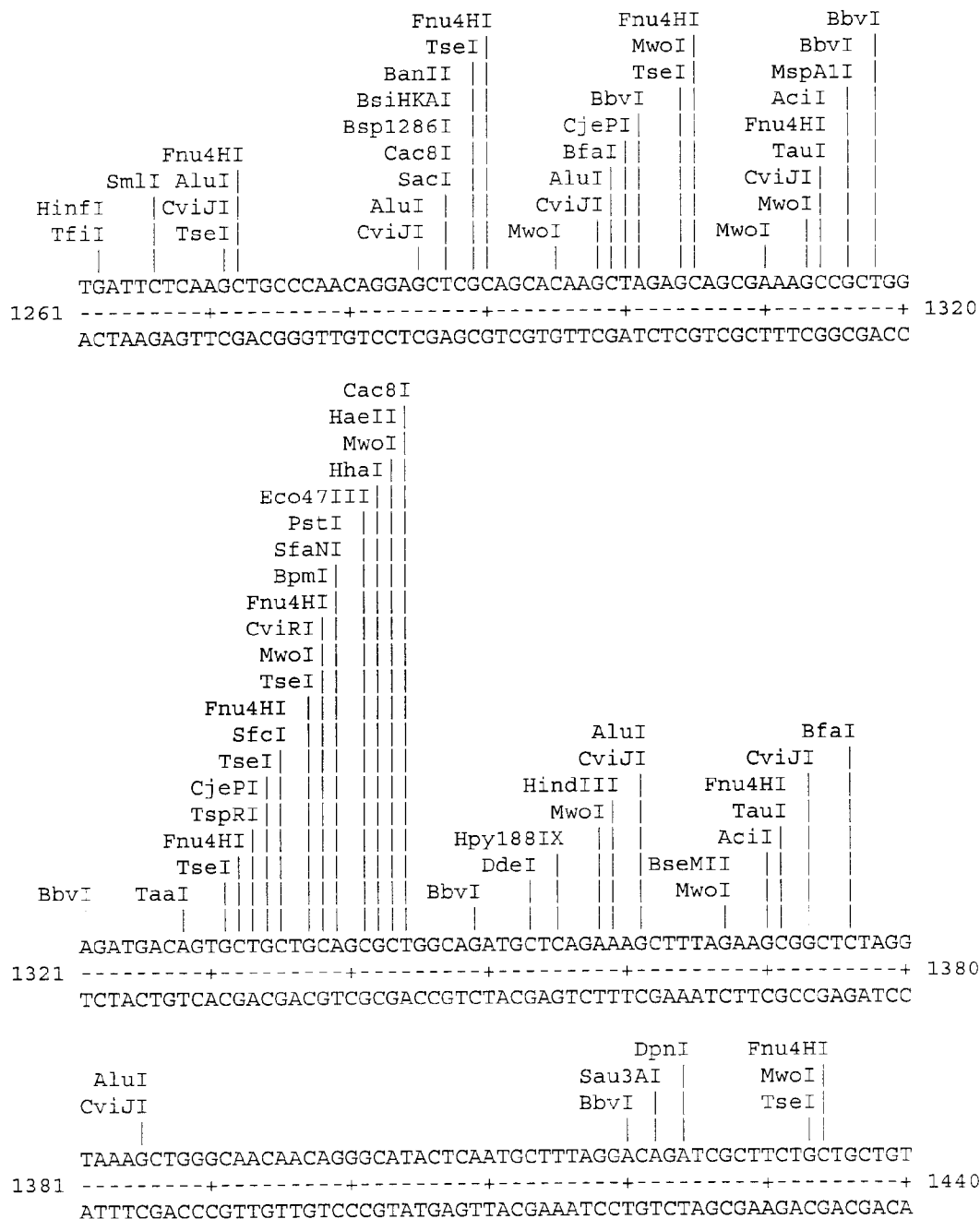
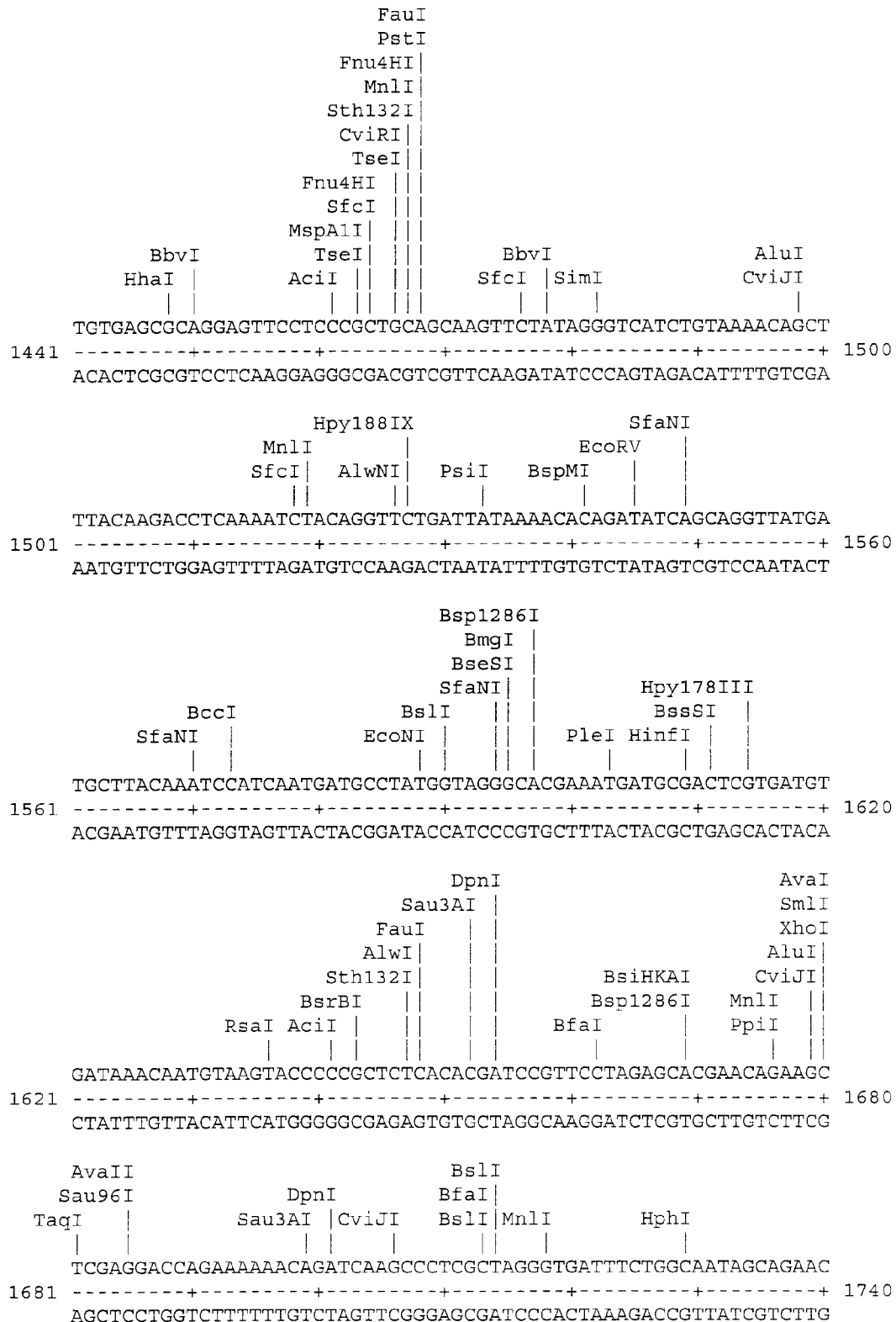


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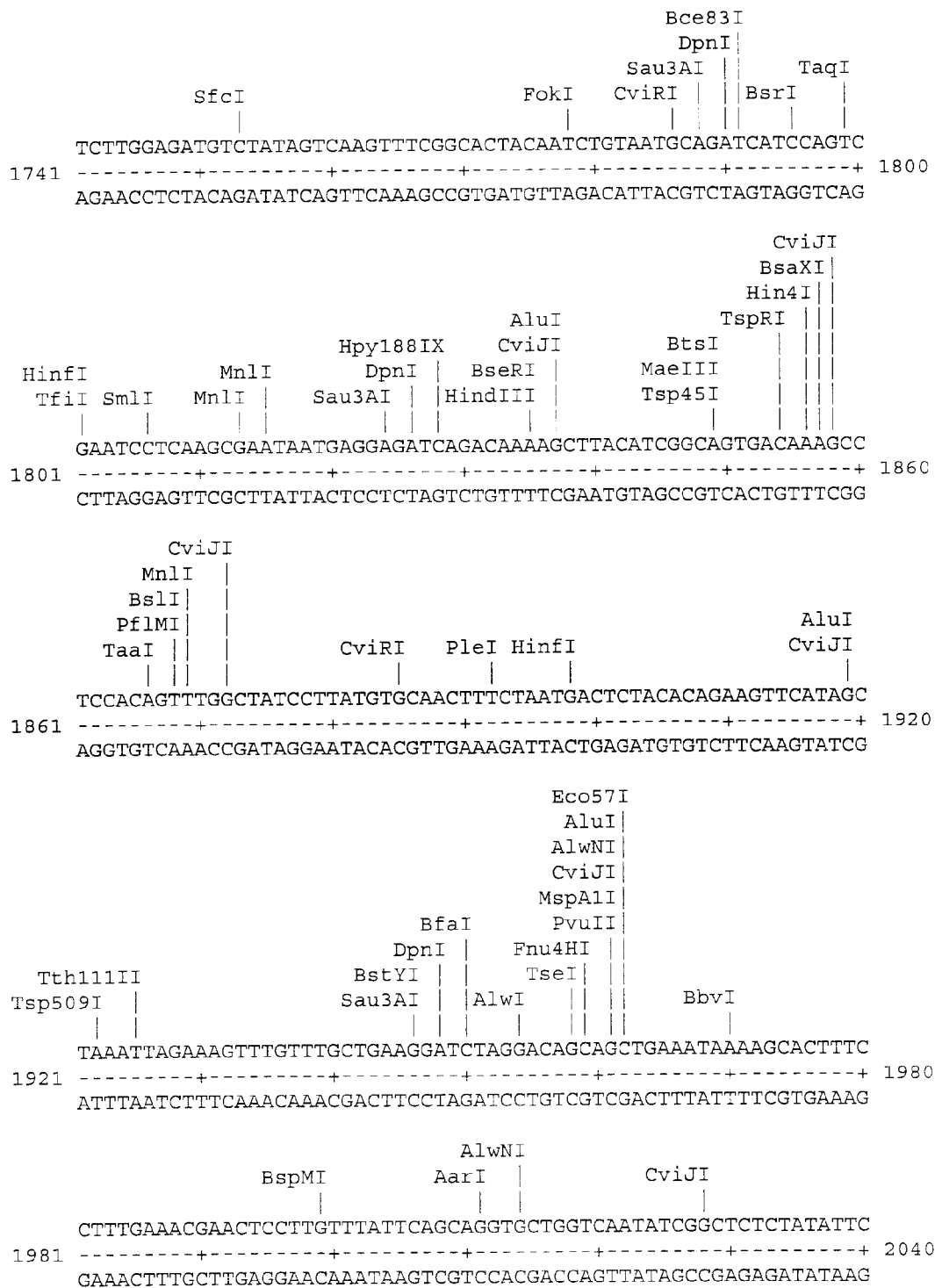
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Figure 2 (continued)



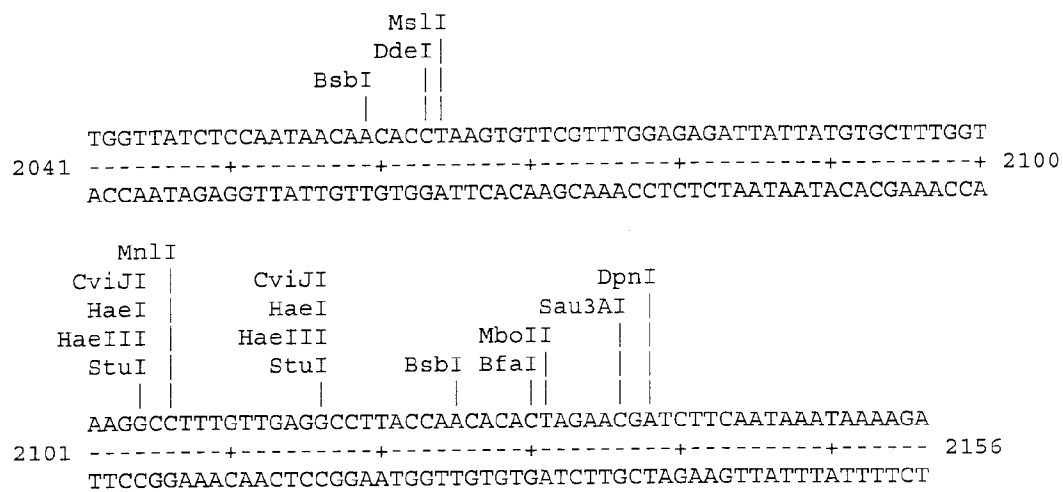
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Figure 2 (continued)



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Figure 2 (continued)



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Figure 3: Sequence Containing Truncated Version of *C. pneumoniae* 76kDa Gene; (nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76kDa Gene).

```

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ctagcttggt ttattggagg aactttaggg ggactcagaa aagctggaga ctggtctgcc 360
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ggcagtgatt ttactttccg aaagtttgat ctaggtataa tttcagcggt ttaagtcaaa 660
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tttttataat aaaactaaaa gattttttatt attttttgag ttttt atg gtt aat cct 777
                                     Met Val Asn Pro
                                     1

att ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat 825
Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp
   5                10                15                20

ctt tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa 873
Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu
          25                30                35

gct caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc 921
Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr
          40                45                50

gat tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc 969
Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu
          55                60                65

atg agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct 1017
Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser
          70                75                80

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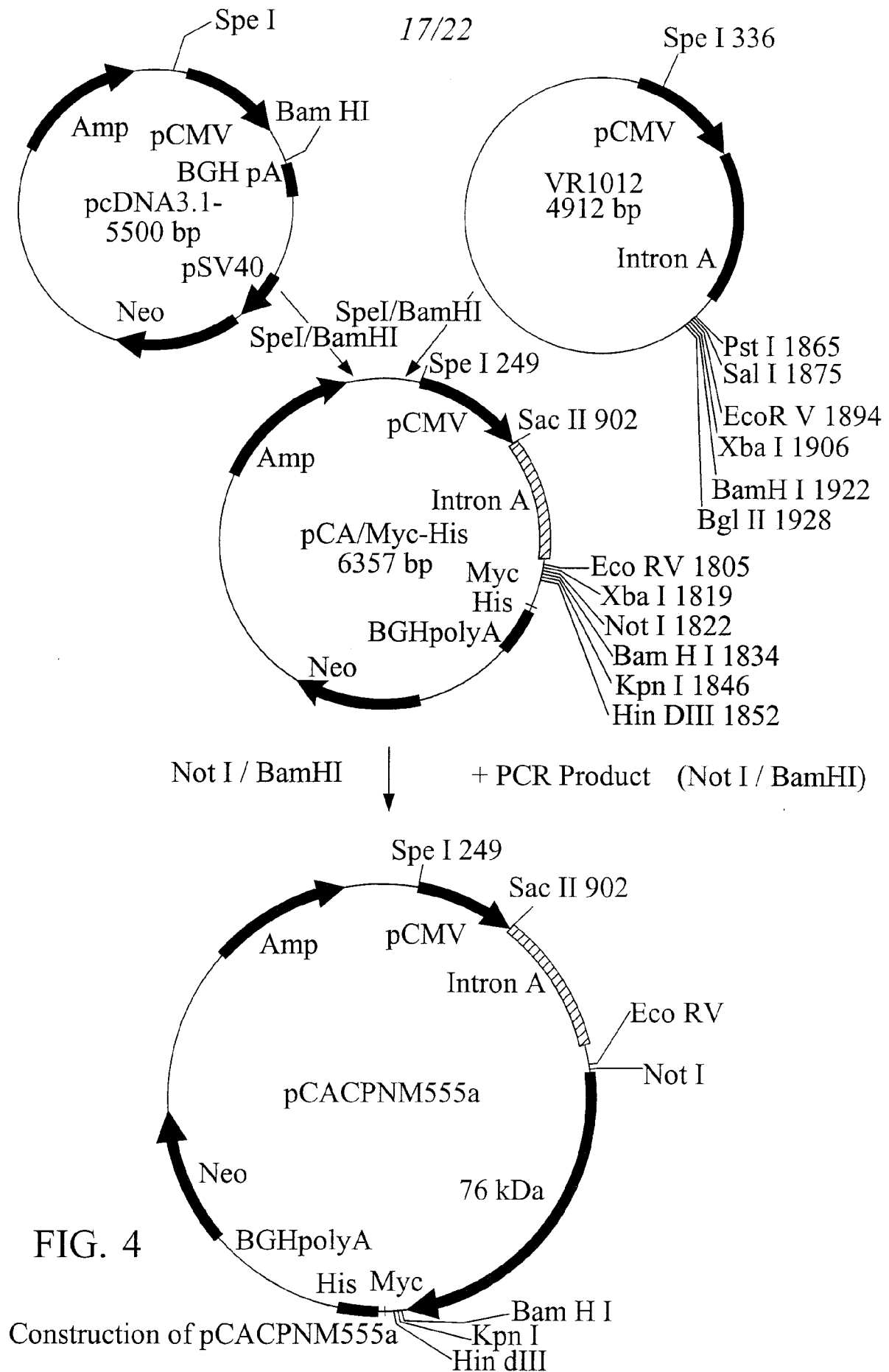
Figure 3 (continued)

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gct tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct	1161
Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala	
120 125 130	
gct ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg	1209
Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala	
135 140 145	
gct act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat	1257
Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn	
150 155 160	
gcc gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat	1305
Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr	
165 170 175 180	
gct tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc	1353
Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser	
185 190 195	
ttc gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac	1401
Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn	
200 205 210	
aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca	1449
Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro	
215 220 225	
ggg aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct	1497
Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala	
230 235 240	
aca gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat	1545
Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr	
245 250 255 260	
ttt gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat	1593
Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn	
265 270 275	
aac agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct	1641
Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala	
280 285 290	
aag aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att	1689
Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile	
295 300 305	

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Figure 3 (continued)

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aat atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca	1785
Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr	
325 330 335 340	
gtt gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt	1833
Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val	
345 350 355	
tcc atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg	1881
Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met	
360 365 370	
tct ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat	1929
Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp	
375 380 385	
tct caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa	1977
Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys	
390 395 400	
gcc gct gga gat gac agt gct gct gca gcg ctg gca gat gct cag aaa	2025
Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys	
405 410 415 420	
gct tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc	2073
Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu	
425 430 435	
aat gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gta	2121
Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val	
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Leu Pro Leu Gln Gln Val Leu Trp Ile Arg Ala Arg Tyr Gln Ala Tyr	
455 460 465	
gta gaa caa aaa ctc atc tca gaa gag gat ctg aat agc gcc gtc gac	2217
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His His His His His	
485 490	



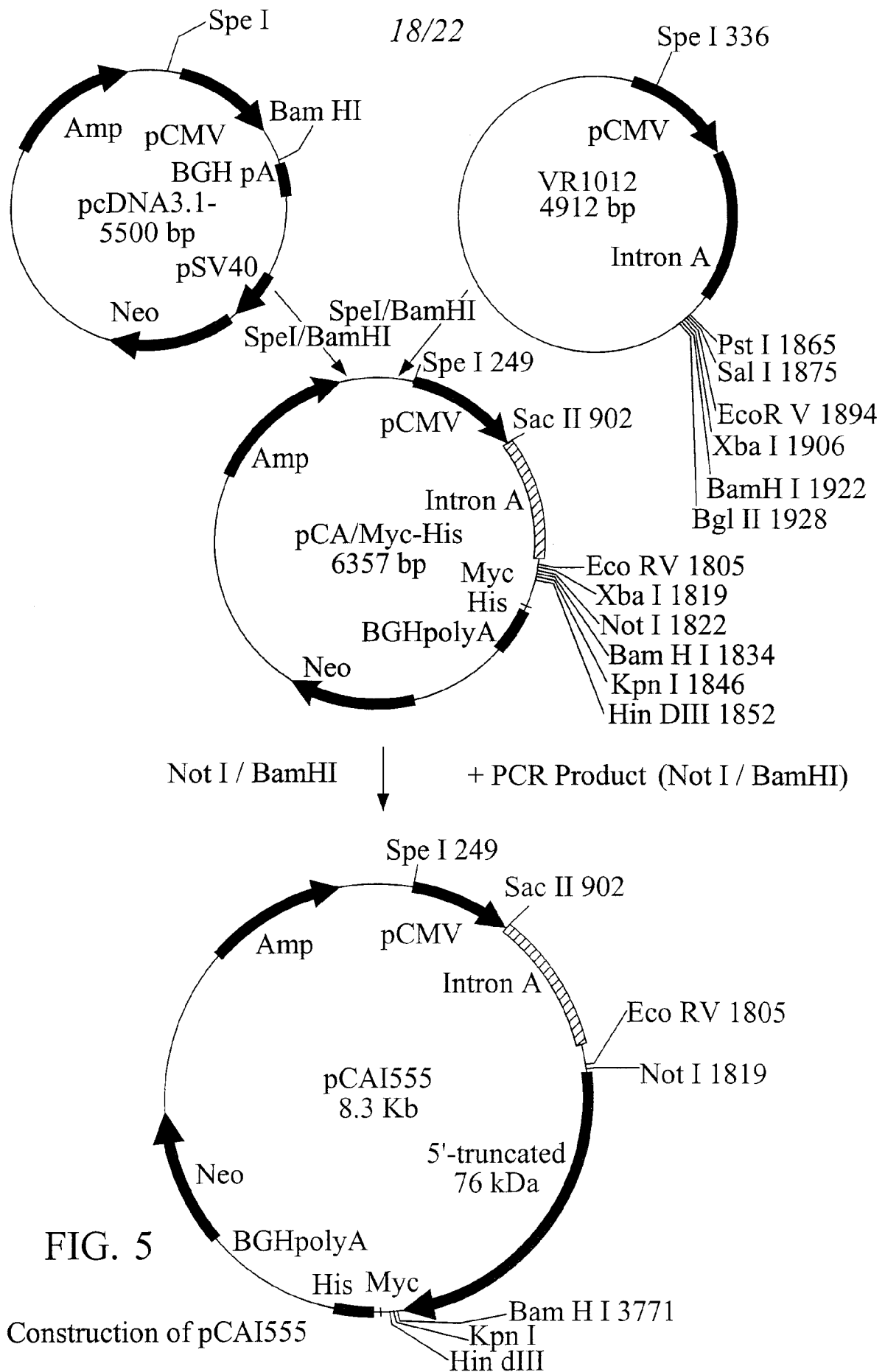


FIG. 5

Construction of pCAI555

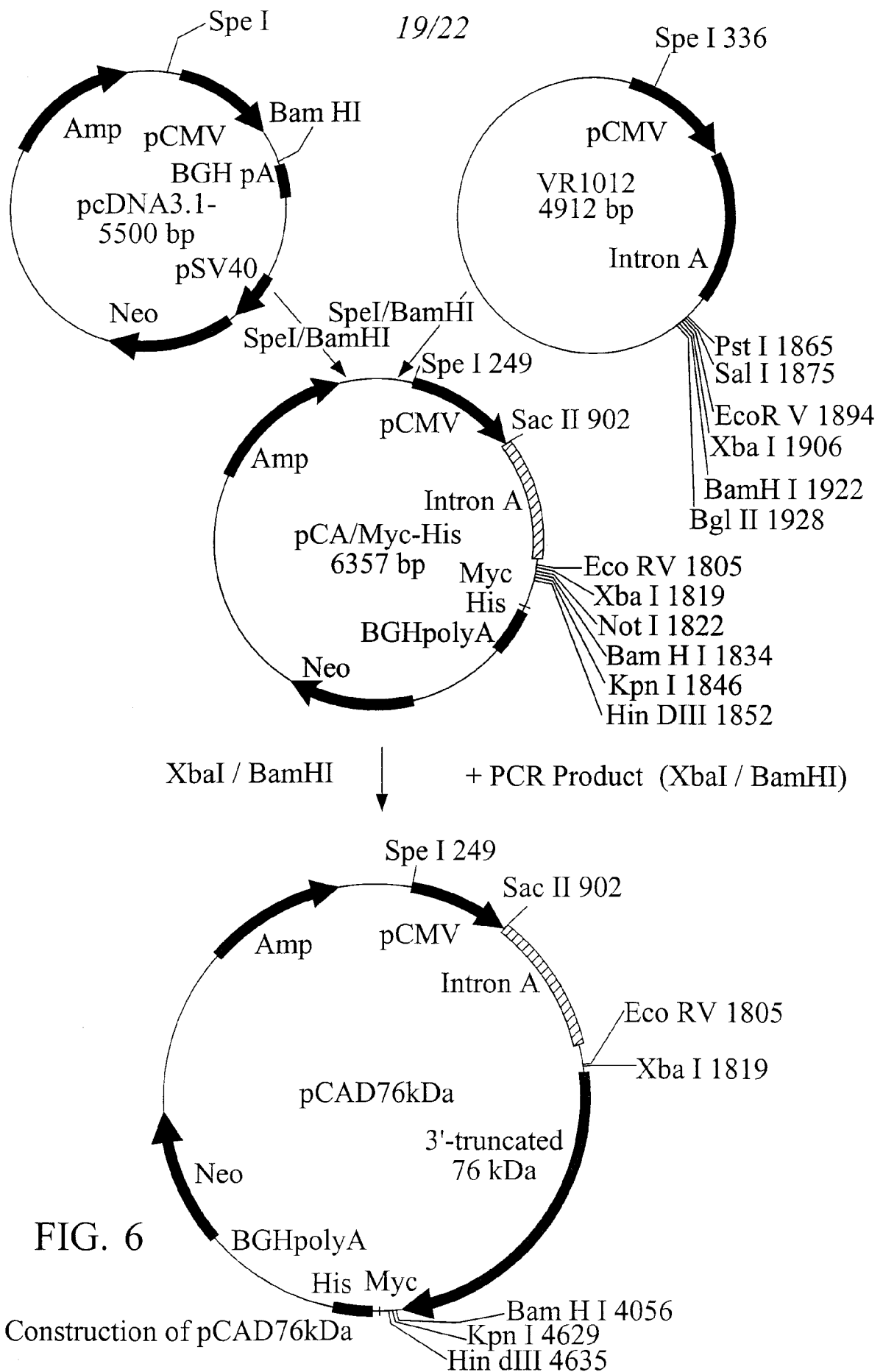


FIG. 6

Construction of pCAD76kDa

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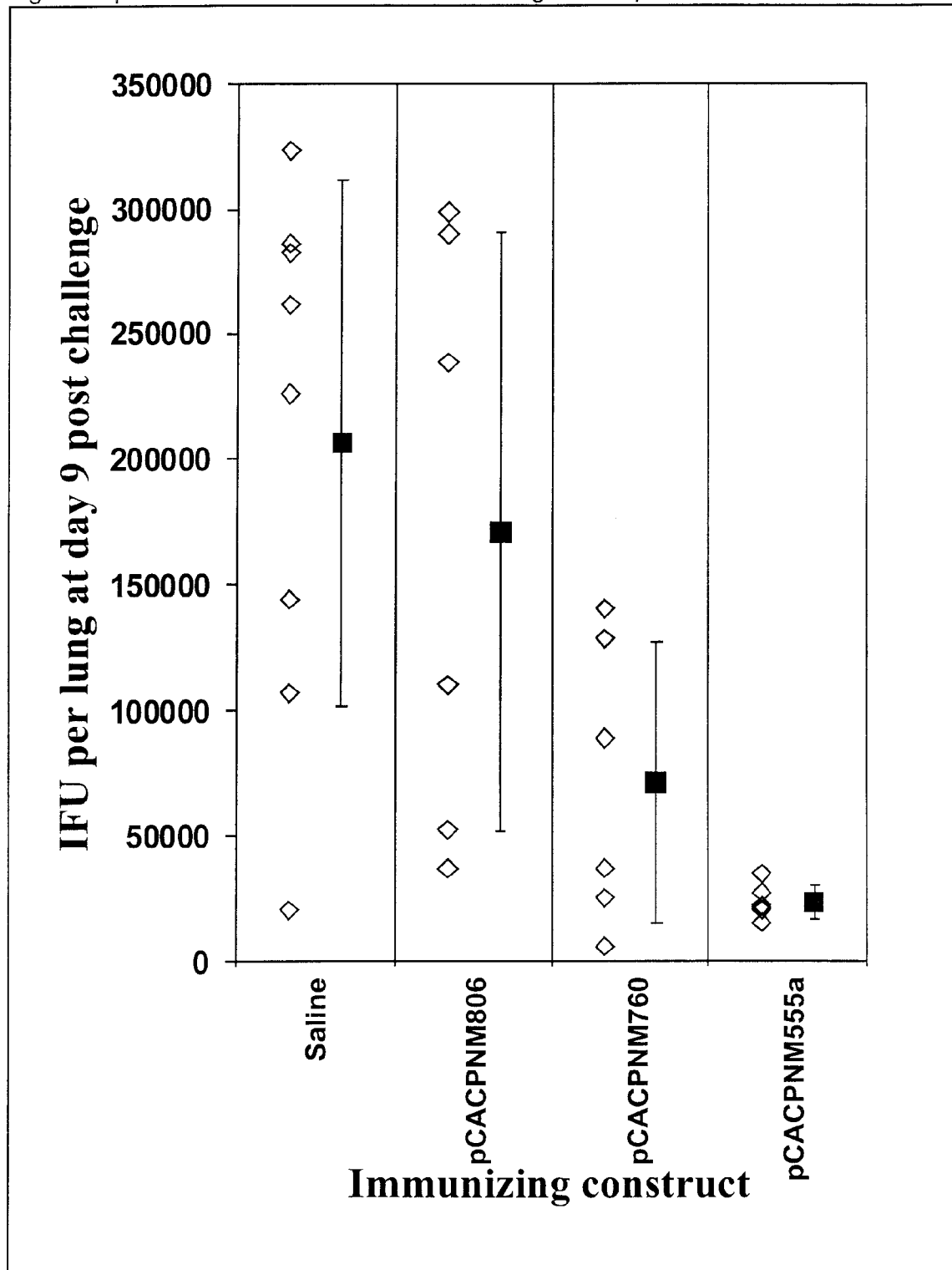
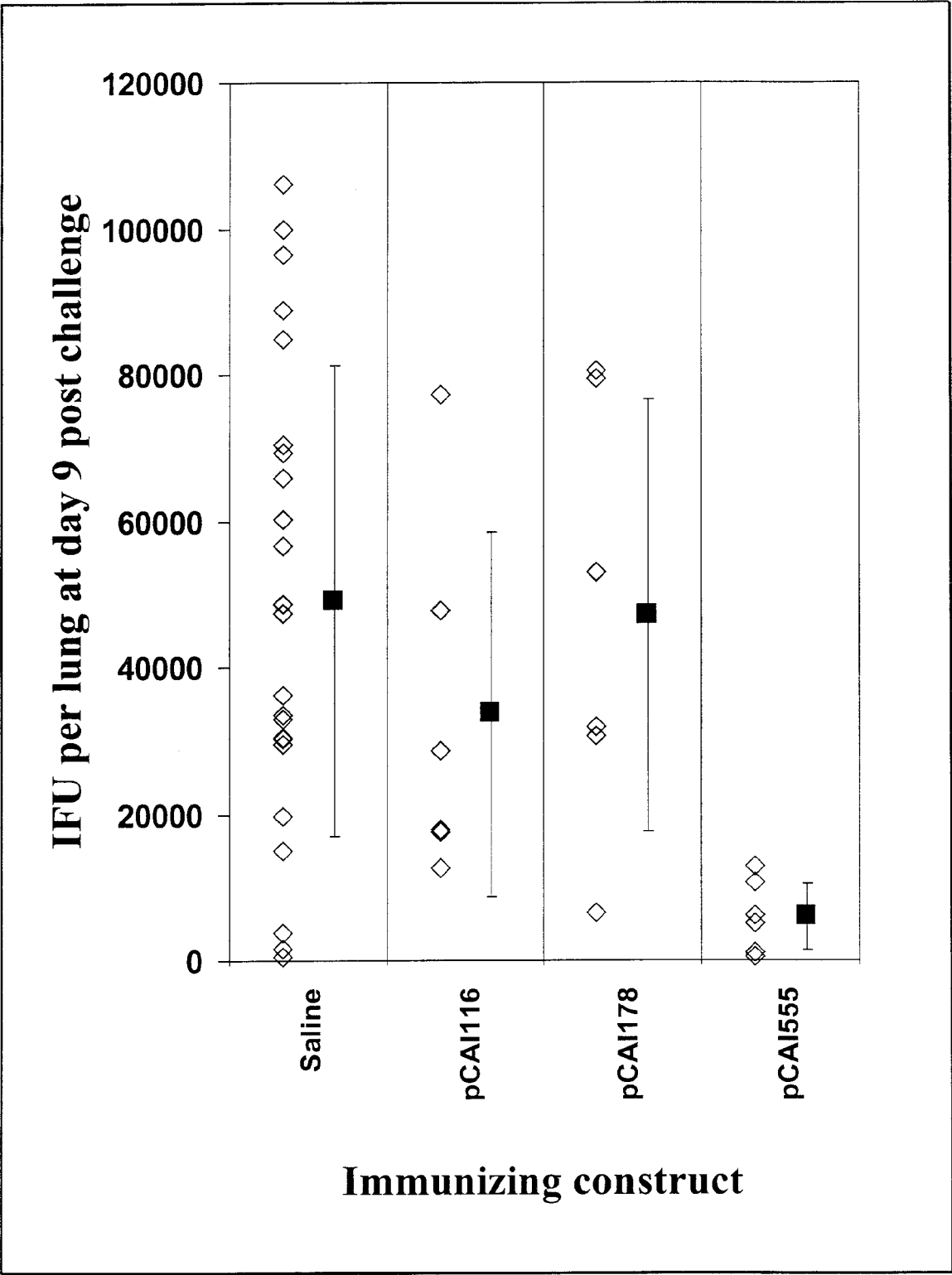
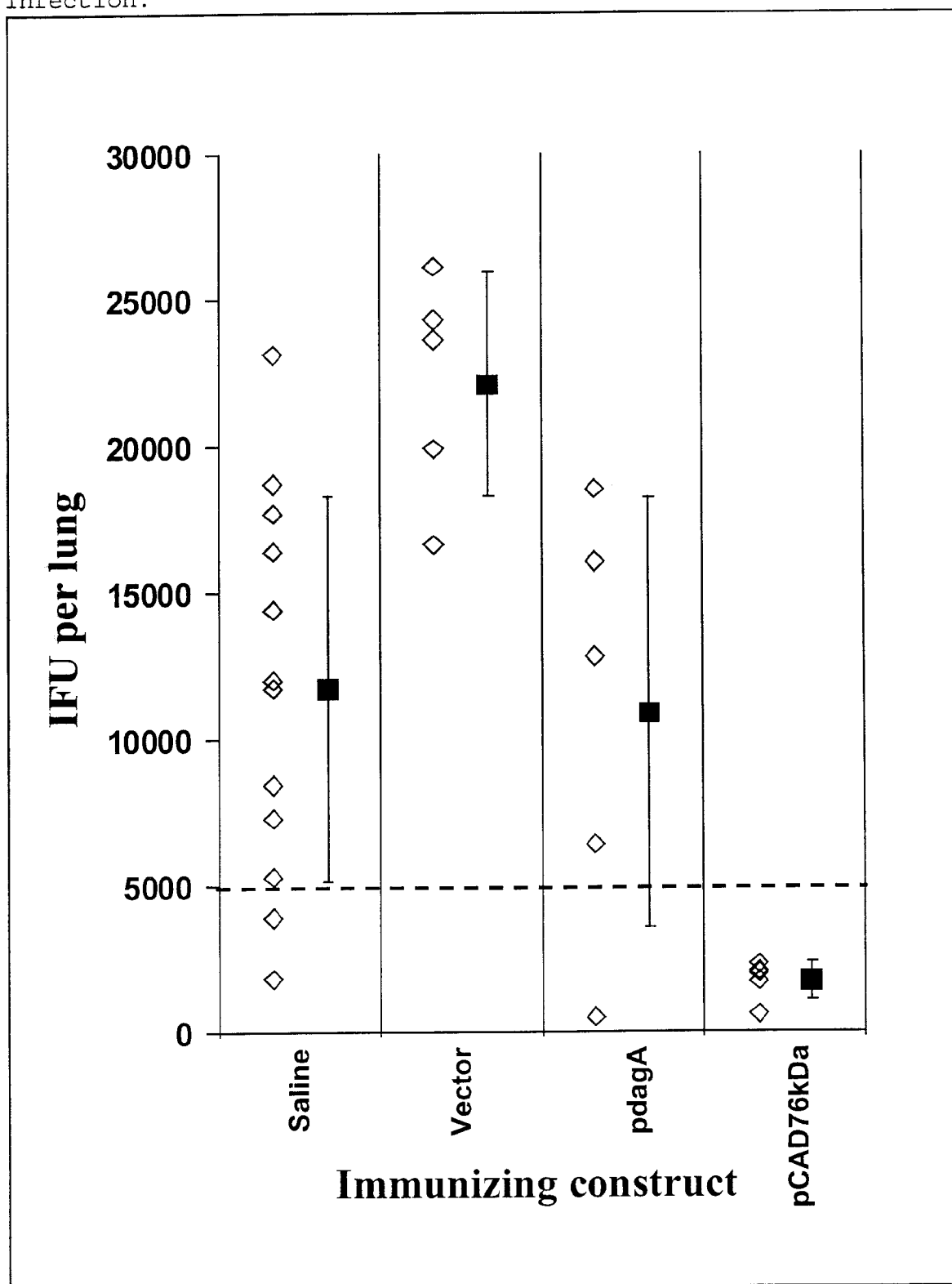
Figure 7: pCACP_{NM555a} Confers Protection Against *C. pneumoniae* Infection .

Figure 8: pCAI555 Confers Protection Against *C. pneumoniae* Infection.



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Figure 9: pCAD76kDa Confers Protection against *C. pneumoniae* Infection.



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Gly	Pro	Gly	Pro	Ile	Asp	Glu	Thr	Glu	Arg	Thr	Pro	Pro	Ala	Asp	Leu	
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Ser	Ala	Gln	Gly	Leu	Glu	Ala	Ser	Ala	Ala	Asn	Lys	Ser	Ala	Glu	Ala	
			25					30					35			

caa	aga	ata	gca	ggt	gcg	gaa	gct	aag	cct	aaa	gaa	tct	aag	acc	gat	259
Gln	Arg	Ile	Ala	Gly	Ala	Glu	Ala	Lys	Pro	Lys	Glu	Ser	Lys	Thr	Asp	
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tct	gta	gag	cga	tgg	agc	atc	ttg	cgt	tct	gca	gtg	aat	gct	ctc	atg	307
Ser	Val	Glu	Arg	Trp	Ser	Ile	Leu	Arg	Ser	Ala	Val	Asn	Ala	Leu	Met	
		55					60				65					

agt	ctg	gca	gat	aag	ctg	ggt	att	gct	tct	agt	aac	agc	tcg	tct	tct	355
Ser	Leu	Ala	Asp	Lys	Leu	Gly	Ile	Ala	Ser	Ser	Asn	Ser	Ser	Ser	Ser	
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90 95 100	
cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca gct	451
Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr Ala	
105 110 115	
tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct gct	499
Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala Ala	
120 125 130	
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Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala Ala	
135 140 145	
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Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn Ala	
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Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr Ala	
170 175 180	
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Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe	
185 190 195	
gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa	739
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys	
200 205 210	
gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca ggg	787
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly	
215 220 225	
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Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr	
230 235 240 245	
gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat ttt	883
Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe	
250 255 260	
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Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn	
265 270 275	
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Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys	
280 285 290	
aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att ctt	1027
Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu	
295 300 305	

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caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat	1075
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Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr Val	
330 335 340	
gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt tcc	1171
Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val Ser	
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390 395 400 405	
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Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys Thr	
520 525 530	

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550 555 560 565

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35 40 45

Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala
50 55 60

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Val	Asn	Ala	Leu	Met	Ser	Leu	Ala	Asp	Lys	Leu	Gly	Ile	Ala	Ser	Ser	
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Asn	Ser	Ser	Ser	Ser	Thr	Ser	Arg	Ser	Ala	Asp	Val	Asp	Ser	Thr	Thr	
				85					90					95		
Ala	Thr	Ala	Pro	Thr	Pro	Pro	Pro	Pro	Thr	Phe	Asp	Asp	Tyr	Lys	Thr	
			100					105						110		
Gln	Ala	Gln	Thr	Ala	Tyr	Asp	Thr	Ile	Phe	Thr	Ser	Thr	Ser	Leu	Ala	
		115					120					125				
Asp	Ile	Gln	Ala	Ala	Leu	Val	Ser	Leu	Gln	Asp	Ala	Val	Thr	Asn	Ile	
	130					135					140					
Lys	Asp	Thr	Ala	Ala	Thr	Asp	Glu	Glu	Thr	Ala	Ile	Ala	Ala	Glu	Trp	
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Glu	Thr	Lys	Asn	Ala	Asp	Ala	Val	Lys	Val	Gly	Ala	Gln	Ile	Thr	Glu	
			165						170					175		
Leu	Ala	Lys	Tyr	Ala	Ser	Asp	Asn	Gln	Ala	Ile	Leu	Asp	Ser	Leu	Gly	
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Lys	Leu	Thr	Ser	Phe	Asp	Leu	Leu	Gln	Ala	Ala	Leu	Leu	Gln	Ser	Val	
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Ala	Lys	Ser	Asn	Asn	Ser	Ile	Ser	Asn	Ile	Asp	Ser	Ala	Lys	Ala	Ala	
		275					280					285				
Ile	Ala	Thr	Ala	Lys	Thr	Gln	Ile	Ala	Glu	Ala	Gln	Lys	Lys	Phe	Pro	
	290					295					300					
Asp	Ser	Pro	Ile	Leu	Gln	Glu	Ala	Glu	Gln	Met	Val	Ile	Gln	Ala	Glu	
305					310					315					320	
Lys	Asp	Leu	Lys	Asn	Ile	Lys	Pro	Ala	Asp	Gly	Ser	Asp	Val	Pro	Asn	
			325						330					335		
Pro	Gly	Thr	Thr	Val	Gly	Gly	Ser	Lys	Gln	Gln	Gly	Ser	Ser	Ile	Gly	
		340						345						350		

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 Ser Ala Gly Val Pro Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val
 450 455 460
 Lys Gln Leu Tyr Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr
 465 470 475 480
 Gln Ile Ser Ala Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr
 485 490 495
 Gly Arg Ala Arg Asn Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser
 500 505 510
 Thr Pro Ala Leu Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg
 515 520 525
 Gly Pro Glu Lys Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn
 530 535 540
 Ser Arg Thr Leu Gly Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser
 545 550 555 560
 Val Met Gln Ile Ile Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile
 565 570 575
 Arg Gln Lys Leu Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr
 580 585 590
 Pro Tyr Val Gln Leu Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys
 595 600 605
 Leu Glu Ser Leu Phe Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys
 610 615 620
 Ala Leu Ser Phe Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val
 625 630 635 640

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Asn Ile Gly Ser Leu Tyr Ser Gly Tyr Leu Gln
 645 650

<210> 3

<211>

<212> DNA

<213> Chlamydia pneumoniae

<220> 1842

<221> CDS

<222> (101..(2053)

<400> 3

atg agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct 48
 Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser
 1 5 10 15

tct act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct 96
 Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro
 20 25 30

acg cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca 144
 Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr
 35 40 45

gct tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct 192
 Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala
 50 55 60

gct ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg 240
 Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala
 65 70 75 80

gct act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat 288
 Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn
 85 90 95

gcc gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat 336
 Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr
 100 105 110

gct tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc 384
 Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser
 115 120 125

ttc gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac 432
 Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn
 130 135 140

aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca 480
 Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro
 145 150 155 160

ggg aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct 528
 Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala
 165 170 175

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aca gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat 576
Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr
                180                      190                      195

ttt gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat 624
Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn
                200                      205                      210

aac agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct 672
Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala
                215                      220                      225

aag aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att 720
Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile
230                      235                      240                      245

ctt caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa 768
Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys
                250                      255                      260

aat atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca 816
Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr
                265                      270                      275

gtt gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt 864
Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val
                280                      285                      290

tcc atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg 912
Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met
                295                      300                      305

tct ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat 960
Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp
310                      315                      320                      325

tct caa gct gcc caa cag gag ctg gca gca caa gct aga gca gcg aaa 1008
Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys
                330                      335                      340

gcc gct gga gat gac agt gct gct gca gcg ctg gca gat gct cag aaa 1056
Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys
                345                      350                      355

gct tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctg 1104
Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu
                360                      365                      370

aat gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gtt 1152
Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val
                375                      380                      385

CCt ccc gct gca gca agt tct ata ggg tca tct gta aaa cag ctg tac 1200
Pro Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val Lys Gln Leu Tyr
390                      395                      400                      405

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aag acc tca aaa tct aca ggt tct gat tat aaa aca cag ata tca gca 1248
Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala
      410                      415                      420

ggt tat gat gct tac aaa tcc atc aat gat gcc tat ggt agg gca cga 1296
Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg
      425                      430                      435

aat gat gcg act cgt gat gtg ata aac aat gta agt acc ccc gct ctc 1344
Asn Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu
      440                      445                      450

aca cga tcc gtt cct aga gca cga aca gaa gct cga gga cca gaa aaa 1392
Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys
      455                      460                      465

aca gat caa gcc ctc gct agg gtg att tct ggc aat agc aga act ctt 1440
Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu
      470                      475                      480                      485

gga gat gtc tat agt caa gtt tcg gca cta caa tct gta atg cag atc 1488
Gly Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile Ile
      490                      495                      500

act cag tcg aat cct caa gcg aat aat gag gag atc aga caa aag ctt 1536
Ile Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu
      505                      510                      515

aca tcg gca gtg aca aag cct cca cag ttt ggc tat cct tat gtg caa 1584
Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln
      520                      525                      530

ctt tct aat gac tct aca cag aag ttc ata gct aaa tta gaa agt ttg 1632
Leu Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu
      535                      540                      545

ttt gct gaa gga tct agg aca gca gct gaa ata aaa gca ctt tcc ttt 1670
Phe Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe
      550                      555                      560                      565

gaa acg aac tcc ttg ttt att cag cag gtg ctg gtc aat atc ggc tct 1718
Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser
      570                      575                      580

cta tat tct ggt tat ctc caa taacaacacc taagtgttcg tttggagaga 1769
Leu Tyr Ser Gly Tyr Leu Gln
      585

ttattatgtg ctttggttaag gcctttgttg aggccttacc aacacactag aacgatcttc 1829

aataaataaaa aga 1842

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<210> 4

<211> 583

<212> PRT

<213> Chlamydia pneumoniae

<400> 4

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Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser
  1              5              10              15

Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro
      20              25              30

Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr
      35              40              45

Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala
      50              55              60

Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala
      65              70              75              80

Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn
      85              90              95

Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr
      100              105              110

Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser
      115              120              125

Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn
      130              135              140

Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro
      145              150              155              160

Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala
      165              170              175

Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr
      180              185              190

Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn
      195              200              205

Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala
      210              215              220

Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile
      225              230              235              240

Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys
      245              250              255

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Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr
 260 265 270
 Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val
 275 280 285
 Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met
 290 295 300
 Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp
 305 310 315 320
 Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys
 325 330 335
 Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys
 340 345 350
 Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu
 355 360 365
 Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val
 370 375 380
 Pro Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val Lys Gln Leu Tyr
 385 390 395 400
 Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala
 405 410 415
 Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg
 420 425 430
 Asn Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu
 435 440 445
 Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys
 450 455 460
 Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu
 465 470 475 480
 Gly Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile
 485 490 495
 Ile Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu
 500 505 510
 Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln
 515 520 525
 Leu Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu
 530 535 540

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Phe Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe
 545 550 555 560

Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser
 565 570 575

Leu Tyr Ser Gly Tyr Leu Gln
 580

<210> 5

<211> 1456

<212> DNA

<213> Chlamydia pneumoniae

<220>

<221> CDS

<222> (101)..(1456)

<400> 5

ataaaatcct taaaaacagg ctgcatttaa ttattagtga gagctttttt tttatttttt 60

ataataaaac taaaagattt ttattatttt ttgagttttt atg gtt aat cct att 115
 Met Val Asn Pro Ile
 1 5

ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat ctt 163
 Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp Leu
 10 15 20

tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa gct 211
 Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu Ala
 25 30 35

caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc gat 259
 Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr Asp
 40 45 50

tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc atg 307
 Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu Met
 55 60 65

agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct tct 355
 Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser Ser
 70 75 80 85

act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct acg 403
 Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro Thr
 90 95 100

cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca gct 451
 Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr Ala
 105 110 115

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tao gat act atc ttt acc tca aca tca cta gct gac ata cag gct gct	499
Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala Ala	
120 125 130	
ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg gct	547
Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala Ala	
135 140 145	
act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat gcc	595
Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn Ala	
150 155 160 165	
gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat gct	643
Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr Ala	
170 175 180	
tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc ttc	691
Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe	
185 190 195	
gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa	739
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys	
200 205 210	
gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca ggg	787
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly	
215 220 225	
aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct aca	835
Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr	
230 235 240 245	
gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat ttt	883
Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe	
250 255 260	
gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat aac	931
Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn	
265 270 275	
agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct aag	979
Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys	
280 285 290	
aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att ctt	1027
Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu	
295 300 305	
caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat	1075
Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn	
310 315 320 325	
atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca gtt	1123
Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr Val	
330 335 340	

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gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt tcc 1171
Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val Ser
      345                      350                      355

atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg tct 1219
Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met Ser
      360                      365                      370

ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat tct 1267
Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp Ser
      375                      380                      385

caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa gcc 1315
Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys Ala
      390                      395                      400                      405

gct gga gat gac agt gct gct gca gcg ctg gca gat gct cag aaa gct 1363
Ala Gly Asp Asp Ser Ala Ala Ala Ala Leu Ala Asp Ala Gln Lys Ala
      410                      415                      420

tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc aat 1411
Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu Asn
      425                      430                      435

gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gta 1456
Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val
      440                      445                      450

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<210> 6

<211> 452

<212> PRT

<213> Chlamydia pneumoniae

<400> 6

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Met Val Asn Pro Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr
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Pro Pro Ala Asp Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn
      20                      25                      30

Lys Ser Ala Glu Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys
      35                      40                      45

Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala
      50                      55                      60

Val Asn Ala Leu Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser
      65                      70                      75                      80

Asn Ser Ser Ser Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr
      85                      90                      95

Ala Thr Ala Pro Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr
      100                      105                      110

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Gln Ala Gln Thr Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala
 115 120 125
 Asp Ile Gln Ala Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile
 130 135 140
 Lys Asp Thr Ala Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp
 145 150 155 160
 Glu Thr Lys Asn Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu
 165 170 175
 Leu Ala Lys Tyr Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly
 180 185 190
 Lys Leu Thr Ser Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val
 195 200 205
 Ala Asn Asn Asn Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn
 210 215 220
 Pro Val Val Pro Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp
 225 230 235 240
 Gln Thr Asp Ala Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile
 245 250 255
 Arg Asp Ala Tyr Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn
 260 265 270
 Ala Lys Ser Asn Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala
 275 280 285
 Ile Ala Thr Ala Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro
 290 295 300
 Asp Ser Pro Ile Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu
 305 310 315 320
 Lys Asp Leu Lys Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn
 325 330 335
 Pro Gly Thr Thr Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly
 340 345 350
 Ser Ile Arg Val Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala
 355 360 365
 Ser Ile Leu Met Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr
 370 375 380
 Glu Asn Pro Asp Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala
 385 390 395 400

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Arg Ala Ala Lys Ala Ala Gly Asp Asp Ser Ala Ala Ala Ala Leu Ala
 405 410 415

Asp Ala Gln Lys Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln
 420 425 430

Gln Gly Ile Leu Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val
 435 440 445

Ser Ala Gly Val
 450

<210> 7
 <211> 2238
 <212> DNA
 <213> Chlamydia pneumoniae

<220>
 <221> CDS
 <222> (766)..(2235)

<400> 7
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 ttttttgtga aatgtagtgt tgtcgactgg aacacattcg ttccttcaga aacctccact 120
 acagaaaaag ctgctacaaa cgctatgaaa taaaaatact gtgtttggca gtggctcgtc 180
 ggaaagcata gtcagggttc ttggatcaat ggacagaaaa agcctctata tctttatgga 240
 gctttcttaa tgaacccttt agcaaaggct acgaagacta cgtaaagtgg aaaagaaaac 300
 ctagcttggt ttattggagg aactttaggg ggactcagaa aagctggaga ctggtctgcc 360
 acagtacgtt atgagtatgt cgaagccttg tcggttccag aaatagatgt ttcagggatt 420
 ggccgtggta atttattaaa gttttggttc gcccaagcaa ttgctgctaa ctatgatcct 480
 aaagaggcta atggttttac aaattataaa ggattttccg ctctatatat gtatggcatc 540
 acagattctc tatcattcag agcttatggg gcttactcca aaccagcaaa cgataaactc 600
 ggcagtgatt ttactttccg aaagtttgat ctaggtataa tttcagcggt ttaagtcaaa 660
 ttttaataaa atcttttaaaa acaggctcgc attaatatt agtgagagct ttttttttat 720
 tttttataat aaaactaaaa gatttttatt attttttgag ttttt atg gtt aat cct 777
 Met Val Asn Pro

1

att ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat 825
 Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp
 5 10 15 20

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ctt tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa	873
Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu	
25 30 35	
gct caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc	921
Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr	
40 45 50	
gat tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc	969
Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu	
55 60 65	
atg agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct	1017
Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser	
70 75 80	
tct act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct	1065
Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro	
85 90 95 100	
acg cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca	1113
Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr	
105 110 115	
gct tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct	1161
Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala	
120 125 130	
gct ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg	1209
Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala	
135 140 145	
gct act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat	1257
Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn	
150 155 160	
gcc gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat	1305
Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr	
165 170 175 180	
gct tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc	1353
Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser	
185 190 195	
ttc gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac	1401
Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn	
200 205 210	
aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca	1449
Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro	
215 220 225	
ggg aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct	1497
Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala	
230 235 240	

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aca Thr 245	gcg Ala	aca Thr	cag Gln	ata Ile	gag Glu 250	aaa Lys	gat Asp	gga Gly	aat Asn	gcg Ala 255	att Ile	agg Arg	gat Asp	gca Ala	tat Tyr 260	1545
ttt Phe	gca Ala	gga Gly	cag Gln	aac Asn 265	gct Ala	agt Ser	gga Gly	gct Ala	gta Val 270	gaa Glu	aat Asn	gct Ala	aaa Lys	tct Ser 275	aat Asn	1593
aac Asn	agt Ser	ata Ile	agc Ser 280	aac Asn	ata Ile	gat Asp	tca Ser	gct Ala 285	aaa Lys	gca Ala	gca Ala	atc Ile	gct Ala 290	act Thr	gct Ala	1641
aag Lys	aca Thr	caa Gln	ata Ile 295	gct Ala	gaa Glu	gct Ala	cag Gln 300	aaa Lys	aag Lys	ttc Phe	ccc Pro	gac Asp 305	tct Ser	cca Pro	att Ile	1689
ctt Leu 310	caa Gln	gaa Glu	gcg Ala	gaa Glu	caa Gln 315	atg Met	gta Val	ata Ile	cag Gln	gct Ala 320	gag Glu	aaa Lys	gat Asp	ctt Leu	aaa Lys	1737
aat Asn 325	atc Ile	aaa Lys	cct Pro	gca Ala 330	gat Asp	ggg Gly	tct Ser	gat Asp	ggt Val	cca Pro 335	aat Asn	cca Pro	gga Gly	act Thr	aca Thr 340	1785
gtt Val	gga Gly	ggc Gly	tcc Ser 345	aag Lys	caa Gln	caa Gln	gga Gly	agt Ser	agt Ser 350	att Ile	ggg Gly	agt Ser	att Ile	cgt Arg 355	gtt Val	1833
tcc Ser	atg Met	ctg Leu	tta Leu 360	gat Asp	gat Asp	gct Ala	gaa Glu	aat Asn 365	gag Glu	acc Thr	gct Ala	tcc Ser	att Ile 370	ttg Leu	atg Met	1881
tct Ser	ggg Gly	ttt Phe	cgt Arg 375	cag Gln	atg Met	att Ile	cac His	atg Met 380	ttc Phe	aat Asn	acg Thr	gaa Glu 385	aat Asn	cct Pro	gat Asp	1929
tct Ser 390	caa Gln	gct Ala	gcc Ala	caa Gln	cag Gln	gag Glu 395	ctc Leu	gca Ala	gca Ala	caa Gln 400	gct Ala	aga Arg	gca Ala	gcg Ala	aaa Lys	1977
gcc Ala 405	gct Ala	gga Gly	gat Asp	gac Asp	agt Ser 410	gct Ala	gct Ala	gca Ala	gcg Ala	ctg Leu 415	gca Ala	gat Asp	gct Ala	cag Gln	aaa Lys 420	2025
gct Ala	tta Leu	gaa Glu	gcg Ala	gct Ala 425	cta Leu	ggg Gly	aaa Lys	gct Ala	ggg Gly 430	caa Gln	caa Gln	cag Gln	ggc Gly	ata Ile 435	ctc Leu	2073
aat Asn	gct Ala	tta Leu	gga Gly	cag Gln 440	atc Ile	gct Ala	tct Ser	gct Ala 445	gct Ala	ggt Val	gtg Val	agc Ser	gca Ala 450	gga Gly	gta Val	2121

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ctc ccg ctg cag caa gtt cta tgg atc cga gct cgg tac caa gct tac 2169
 Leu Pro Leu Gln Gln Val Leu Trp Ile Arg Ala Arg Tyr Gln Ala Tyr
 455 460 465

gta gaa caa aaa ctc atc tca gaa gag gat ctg aat agc gcc gtc gac 2217
 Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp
 470 475 480

cat cat cat cat cat cat tga 2238
 His His His His His His
 485 490

<210> 8

<211> 490

<212> PRT

<213> Chlamydia pneumoniae

<400> 8

Met Val Asn Pro Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr
 1 5 10 15

Pro Pro Ala Asp Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn
 20 25 30

Lys Ser Ala Glu Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys
 35 40 45

Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala
 50 55 60

Val Asn Ala Leu Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser
 65 70 75 80

Asn Ser Ser Ser Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr
 85 90 95

Ala Thr Ala Pro Thr Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr
 100 105 110

Gln Ala Gln Thr Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala
 115 120 125

Asp Ile Gln Ala Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile
 130 135 140

Lys Asp Thr Ala Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp
 145 150 155 160

Glu Thr Lys Asn Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu
 165 170 175

Leu Ala Lys Tyr Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly
 180 185 190

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Lys Leu Thr Ser Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val
 195 200 205

Ala Asn Asn Asn Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn
 210 215 220

Pro Val Val Pro Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp
 225 230 235 240

Gln Thr Asp Ala Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile
 245 250 255

Arg Asp Ala Tyr Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn
 260 265 270

Ala Lys Ser Asn Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala
 275 280 285

Ile Ala Thr Ala Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro
 290 295 300

Asp Ser Pro Ile Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu
 305 310 315 320

Lys Asp Leu Lys Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn
 325 330 335

Pro Gly Thr Thr Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly
 340 345 350

Ser Ile Arg Val Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala
 355 360 365

Ser Ile Leu Met Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr
 370 375 380

Glu Asn Pro Asp Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala
 385 390 395 400

Arg Ala Ala Lys Ala Ala Gly Asp Asp Ser Ala Ala Ala Ala Leu Ala
 405 410 415

Asp Ala Gln Lys Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln
 420 425 430

Gln Gly Ile Leu Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val
 435 440 445

Ser Ala Gly Val Leu Pro Leu Gln Gln Val Leu Trp Ile Arg Ala Arg
 450 455 460

Tyr Gln Ala Tyr Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
 465 470 475 480

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Ser Ala Val Asp His His His His His His
485 490

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<211> 43
<212> DNA
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<400> 9

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<211> 35
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<210> 12
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<212> DNA
<213> primer

<400> 12

gcgccggatc ccttggagat aaccagaata ta 32

<210> 13
<211> 38
<212> DNA
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<400> 13

gctctagacc gccatgacaa aaaaacatta tgcttggg 38

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<210> 14
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<400> 14

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28